ORIGINAL ARTICLE

Allosensitization after transplant failure: the role of graft nephrectomy and immunosuppression – a retrospective study

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SUMMARY

There are conflicting data about the role of transplant nephrectomy and immunosuppression withdrawal on the development of allosensitization and the impact on re-transplantation. We divided 109 first graft recipients into two groups according to whether they underwent nephrectomy (NX+, $n = 61$) or their graft was left *in situ* (NX−, $n = 48$). Sera were assessed for HLA-A/B/Cw/DR/DQ antibodies at the time of NX/transplant failure and after 3, 6, 12, 24 months. The NX+ group showed a higher rate of donor specific antibody (DSA) and non-DSA human leukocyte antigen (HLA) antibody production at all the time points. Multivariable analysis showed that nephrectomy was a strong, independent risk factor for the development of DSAs after 12 and 24 months ($P = 0.005$ and 0.008). In the NX− group, low tacrolimus levels correlated with DSA formation (AUC 0.817, $P = 0.002$; best cut-off level 2.9 ng/ml). Analysis with a standardized pool of UK donors showed a more difficult grade of HLA matchability following nephrectomy compared with the NX− group. Nephrectomy is followed by the long-term production of DSA and non-DSA HLA antibodies and negatively impacts on the chances of finding a HLA-compatible kidney. Tacrolimus levels ≥3 ng/ml are protective against the development of allosensitization and could facilitate re-transplantation in the NX− group.

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Key words
allosensitization, donor-specific antibody, tacrolimus, transplant nephrectomy

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Introduction

The number of patients wait listed for renal transplantation continues to increase [1], as does the incidence of patients returning to dialysis after graft failure [2]. In this group of patients re-transplantation has been shown to reduce the mortality rate by up to 45% [3–5]. Patients returning to dialysis after transplant failure often develop human leukocyte antigen (HLA) antibodies, which may preclude the opportunity of receiving a further transplant [6] and are strongly associated with an increased risk of acute rejection and graft loss after re-transplantation [7,8]. Allograft nephrectomy is a known event leading to allosensitization and the formation of donor-specific antibodies (DSA) [9,10]. The reasons for this are not fully understood. The role of withdrawal of immunosuppression after returning to dialysis or following allograft nephrectomy has been examined with conflicting results [11–13].

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There is therefore no consensus regarding the optimal management of the patient returning to dialysis after graft failure with respect to allograft nephrectomy and the continuation of immunosuppression. Furthermore, there are no large studies assessing the long-term effects of allograft nephrectomy and of other potential risk factors for the development of allosensitization in patients with a first failed graft not previously significantly sensitized. Although it has been suggested that patients undergoing transplant nephrectomy might remain waitlisted for re-transplantation longer than patients who had the graft left *in situ* [14], to our knowledge, there are no studies investigating the chances of finding a HLA-compatible kidney.

In this retrospective, single-center study in patients with a first failed graft returning to dialysis, we compare the tempo and class of donor and non-donor directed HLA antibody development up to 24 months after allograft failure in a group of patients who underwent transplant nephrectomy with a group of patients who had the graft maintained *in situ*. We also identify the risk factors for the development of subsequent allosensitization and focus on the potential role of immunosuppression withdrawal. Finally, we assess the likelihood of re-transplantation in the two study groups.

Methods

Patients

This study was approved by the Imperial College Renal and Transplant Research Group. About 1714 patients received a renal transplant at our center between August 2004 and October 2015. Two hundred and sixty-three (15.3%) returned to dialysis, and of these 106 (40.3%) underwent transplant nephrectomy. In order to reduce possible bias due to the allosensitization induced by multiple failed transplants, we selected recipients of a first graft with no DSAs detectable at the time of nephrectomy and transplant failure. One hundred and nine patients were thus included in the study and divided into two groups according to whether they underwent allograft nephrectomy (NX+, $n = 61$) or had the graft left *in situ* (NX−, $n = 48$). The indications for nephrectomy were thrombosis/hemorrhage (29/61; 47.5%), infection (14/61; 22.9%), graft intolerance syndrome (9/61; 14.8%), other (9/61; 14.8%). All nephrectomies were sub-capsular.

Immunosuppression

At the time of transplant, all patients were CDC and flow cross-match negative. Patients received induction with either the anti-CD52 monoclonal antibody Alemtuzumab (Campath-1H; Genzyme, Oxford, UK) or the anti-IL2 receptor monoclonal antibody (Daclizumab; Nutley, Roche Inc, NJ, USA or Basiliximab; Novartis Pharma Corp, East Hanover, NJ, USA). Patients treated with Alemtuzumab received tacrolimus monotherapy and patients treated with Daclizumab/Basiliximab received both tacrolimus and mycophenolate mofetil (MMF). All patients received a steroid sparing protocol with 500 mg methylprednisolone perioperatively followed by corticosteroids for only the first postoperative week.

Following allograft failure, MMF was stopped when dialysis was restarted. Tacrolimus was discontinued at the time of nephrectomy in 50/61 patients who underwent surgery before 2011, whereas 11/61 patients had the tacrolimus tapered down within an average period of 120 days after surgery and then stopped. NX− patients were maintained on tacrolimus if not otherwise contraindicated and the dose was reduced by approximately 50%. At the time of nephrectomy, 23/61 (37.7%) patients were on low dose prednisolone and steroids were subsequently stopped in 11 of these patients within the first 9 months after nephrectomy. In the NX−, 20/48 (41.7%) were on low dose prednisolone at the time of graft failure.

HLA antibody analysis

All recipients and donors were typed for HLA-A/-B/-C, HLA-DRB1/B3/B4/B5 and HLA-DQB1 loci using in house PCR-SSP (sequence-specific primers) or the LABType® SSO typing kits (One Lambda, Canoga Park, CA, USA). Sera were tested for class I HLA (-A/-B/-Cw) and class II (-DR/-DQ) antibodies at the time of nephrectomy or graft failure and after 3, 6, 12, and 24 months using the single antigen Luminex assay performed with LABScreen single antigen class I and II beads (One Lambda) according to the manufacturer's instructions. In order to compare mean fluorescence intensity (MFI) values of sera that were found to be saturating in the Luminex assay because of the presence of high antibody titre (MFI > 15 000), sera were further tested and analyzed by either serum dilution (1:2 dilution with Human AB sera) or EDTA treatment (1:2 dilution with Luminex wash buffer containing 6% EDTA). In serum samples that had high titre antibody and were possibly saturating in the assay, the reported MFI values were not further adjusted to account for the dilution factor. We considered positive an MFI value above 1000 to minimize the risk of overestimating the degree of sensitization because of transient, low level

HLA antibodies [15]. Antibody specificity was reported as equivalent to those defined by serological methods (https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/2926/hla_unacceptable_antigen_mapping.pdf). The number of HLA antibodies reported corresponded to the number of different unacceptable antigens detected by the single antigen test and not the cumulative number of positive beads of the same HLA molecule.

Panel Reactive Antibody and transplant matchability

The degree of allosensitization, expressed as percent Panel Reactive Antibody (PRA%) for HLA class I, class II, or both, and transplant matchability was calculated using the calculator supplied by the Organ Donation and Transplantation UK (www.odt.nhs.uk). Matchability estimates the relative ease or difficulty a patient may have in finding a HLA-compatible kidney within the general donor population and was calculated from a standardized pool of 10 000 UK donors taking into account blood group, HLA type and unacceptable class I and II HLA antigens (-A/-B/-Cw/-DR/-DQ). Transplant matchability is expressed with a score 1 to 10 and according to three matching categories: easy (1–3), moderate (4–7) or difficult (8–10).

Statistical analysis

Normally distributed variables were compared with Student's *t* test or chi-squared/Fisher's exact test. Non-parametric data were compared with Mann-Whitney, Wilcoxon's, or Kruskal-Wallis test as appropriate. Friedman's and Cochran's *Q* test were used to compare repeated measures within the same group of patients. Univariable and multivariable logistic regression models were used to analyze the variables potentially associated with DSA positivity. Data are reported as mean ± standard deviation or median and interquartile range as appropriate. Statistical and graphical analyses were performed with IBM SPSS STATISTICS Ver. 20.0 and GRAPHPAD PRISM Ver. 6.0, respectively. The two-sided level of significance was set at $P < 0.05$.

Results

Patients

Table 1 summarizes the patients demographics. There were no significant differences in the ethnicities, blood groups, and length of follow-up ($P = 0.234$, 0.896 and, 0.280 respectively) between the NX+ and NX−. The median time from graft failure to nephrectomy was 0 (0–86) days. No patients died within the 24-month study period. Both groups were equally transfused before transplant failure or nephrectomy, whereas patients in the NX+ were transfused more at the time or after nephrectomy compared with the NX−. There was no difference in the induction regimens between the NX+ and NX− except nine (14.8%) NX+ patients who lost their graft at the time of implantation or just after surgery and therefore did not receive induction.

De novo DSA production

Starting from 3 months after nephrectomy we observed a rising proportion of patients developing at least one DSA (47.7%, 64.9%, 83.0%, 85.2% at 3, 6, 12, 24 months respectively, $P < 0.001$ for all time points compared with NX−). In the NX−, DSA production was less and delayed (10.3%, 24.4%, 41.0% at 6, 12, 24 months respectively, Fig. 1). The proportion of patients who remained DSA negative but developed HLA antibodies [cumulative HLA class I + II Panel Reactive Antibody (cPRA%) >25%] did not significantly change with time between and within groups. The above findings were confirmed even after the exclusion of the patients who experienced graft loss within 3 months from transplant (29 in the NX+ and 2 in the NX−, $P < 0.001$ for all time points).

There was a higher prevalence of class II only positive patients in the NX− at 24 months, whereas the prevalence of class I only DSA-positive and class I+II DSA-positive patients was higher in the NX+ (Fig. 2). This rose with time in both groups ($P < 0.001$ and 0.003 for trend, NX+ and NX− respectively) and was significantly higher in the NX+ at 6, 12, and 24 months compared with the NX− (Fig. 3).

At 24 months, the median MFI value of the immunodominant class I DSA in the NX+ was significantly higher compared with the NX− [9961 (5289–14 184) vs. 5111 (2793–8622), $P = 0.027$], as well as the cumulative MFI for the class I DSAs [18 107 (9612–29 061) vs. 8206 (2793–14 320), $P = 0.031$]. However, there was no difference between the class II DSAs [immunodominant MFI 9879 (2756–13 640) vs. 8743 (2450–14 877), NX+ and NX− respectively, $P = 0.785$; cumulative MFI 14 030 (2760–21 370) vs. 10 929 (3754–17 034), NX+ and NX− respectively, $P = 0.595$].

In the NX+, the timing of surgery did not change the tempo and prevalence of subsequent DSA production. We divided this cohort into three subgroups according to the timing they had the nephrectomy: within 24 h

Table 1. Patient characteristics.

	NX$^+$ (n = 61)	NX$^-$ (n = 48)	P
Female, n (%)	23 (37.7)	18 (37.5)	0.983
Age at failure, years	48 ± 15	54 ± 12	0.036
Previous pregnancy, n (%)	14 (23.0)	8 (16.7)	0.661
Blood products transfusions, n (%)	58 (95.1)	45 (93.8)	0.762
Bloods products exposure			
Red blood cells	60 (98.4)	45 (93.8)	0.318
Platelets	33 (54.1)	21 (43.8)	0.336
Plasma/cryoprecipitate	34 (55.7)	18 (37.5)	0.082
Transfused before Nx/failure, n (%)	49 (80.3)	43 (89.6)	0.287
Transfused after Nx/failure, n (%)	55 (90.2)	28 (58.3)	<0.001
Transfusion events before Nx/failure	4.0 (1.0–11.0)	4.0 (2.0–8.0)	0.695
Transfusion events after Nx/failure	4.0 (2.0–7.0)	1.0 (0.0–2.0)	<0.001
Number of transfusion events after Nx/failure per month	0.17 ± 0.12	0.06 ± 0.08	<0.001
Pre-emptive transplant, n (%)	14 (23.0)	11 (22.9)	0.997
Living donor transplant, n (%)	26 (42.6)	17 (35.4)	0.554
SPK transplant, n (%)	1 (1.6)	7 (14.6)	0.021
Donor age, years	53 ± 14	51 ± 14	0.485
Induction			
Anti-CD52, n (%)	40 (65.6)	38 (79.2)	0.020
Anti-IL2 receptor, n (%)	12 (19.7)	10 (20.8)	
No induction, n (%)	9 (14.8)	0 (0.0)	
History of rejection, n (%)	18 (29.5)	12 (25.0)	0.669
Pre-transplant RRT, months	18.8 (0.1–79.1)	19.8 (1.3–64.5)	0.927
Transplant duration, days	111 (4–957)	1764 (860–2465)	<0.001
Causes of graft failure, n (%)			
Thrombosis/hemorrhage	32 (52.5)	4 (8.3)	<0.001
Rejection	14 (23.0)	8 (16.7)	
Infection	3 (4.9)	7 (14.6)	
GN/recurrent disease	3 (4.9)	11 (22.9)	
Scarring/other	9 (14.8)	18 (37.5)	
A/B/Cw mismatch	3.6 ± 1.4	3.4 ± 1.8	0.947
DR/DQ mismatch	1.7 ± 1.1	1.8 ± 1.3	0.893
A/B/Cw/DR/DQ mismatch	5.3 ± 2.0	5.1 ± 2.6	0.819
Immunosuppression regimen			
Tac monotherapy, n (%)	30 (49.2)	19 (39.6)	0.339
Tac + MMF, n (%)	31 (50.8)	29 (60.4)	

MMF, mycophenolate mofetil; NX, nephrectomy; SPK, simultaneous pancreas-kidney; Tac, tacrolimus.

Data are presented as number (%), mean ± standard deviation or median (interquartile range).

from implantation (n = 13), 1–30 days from transplant failure (n = 27) and >30 days after transplant failure (n = 21). We found no difference in the prevalence of the DSA-positive patients with time (P = 0.445, 0.164, 0.996, and 0.746 at 3, 6, 12, and 24 months, respectively, Fig. 4).

Breadth of HLA class I and II specificities

The median number of HLA class I antibodies rose significantly with time in the NX+ but not in the NX− (P < 0.001 and P = 0.064 respectively), whereas the median number of HLA class II antibody specificities was increased with time in both groups (P < 0.001 and P = 0.002, NX+ and NX− respectively). The median number of both HLA class I and II antibody specificities was higher in the NX+ compared with the NX− at all the time points (Fig. 5). Table 2 similarly shows a rising PRA% in both study groups with time, with class I PRA% being higher in the NX+ compared with the NX− at all time points. Class II PRA% was higher in the NX+ at 3, 6, 12 months but not at 24 months. No difference in the number of specificities and PRA% was found across the three nephrectomy timing subgroups.

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Risk factors for the development of DSAs

We wanted to identify the potential risk factors for the development of DSAs after 12 and 24 months from nephrectomy or transplant failure. Univariable analysis did not show significant correlations between DSA formation and the following variables: gender, pre-emptive transplant, deceased-donor/live-donor transplant, donor age, immunosuppression induction. With particular regard to the latter, subgroup analysis did not show any effect of the lack of immunosuppression induction on the development of DSAs, confirming previous reports [16]. We performed a multivariable logistic regression analysis including the classical risk factors for DSA production (HLA mismatch, previous rejection, allograft nephrectomy) and allosensitization in transplant and end stage renal disease patients (pregnancy and blood transfusions) [17,18]. We also included the transplant duration in the present model given the significant difference between the NX+ and NX− cohorts. At 12 months, univariable analysis showed that transplant nephrectomy, blood transfusions, and previous rejection were associated with the development of DSAs, whereas transplant duration was a protective factor. After multivariable analysis, transplant nephrectomy and previous rejection remained the only significant risk factors for the development of DSAs. Transplant duration showed again to be a protective factor, although the correlation did not appear to be strong (Table 3). Univariable analysis at 24 months showed similar results. After multivariable analysis only transplant nephrectomy remained a strong risk factor, whereas previous rejection and HLA mismatch were significant, albeit weaker, risk factors (Table 4).

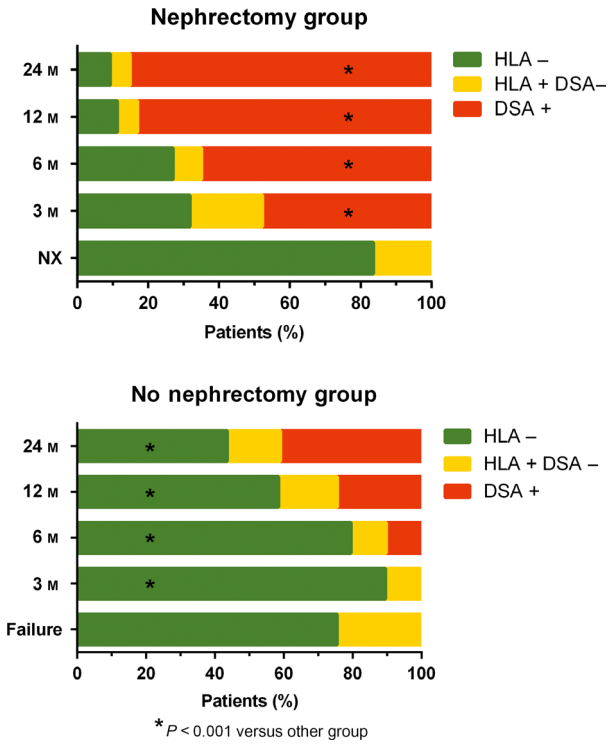

Figure 1 Changes of the allosensitization status with time in the nephrectomy and no-nephrectomy (NX−) group.

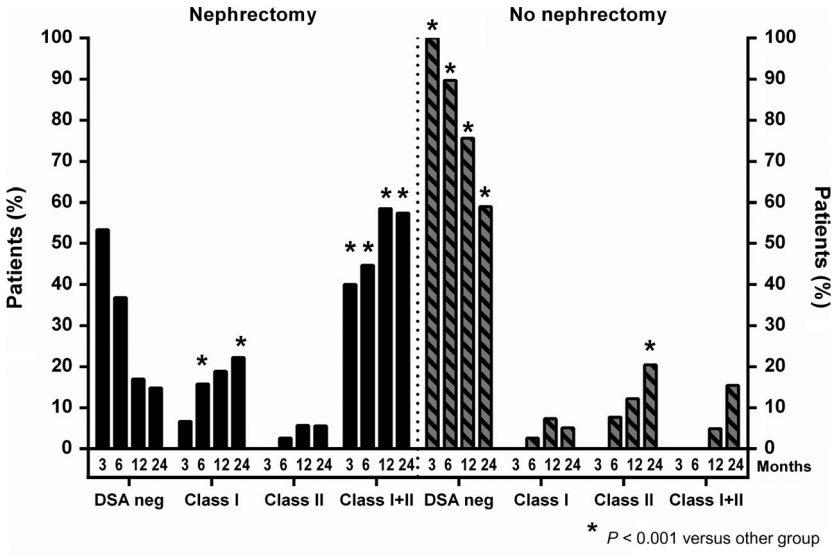

Figure 2 Prevalence of donor-specific antibody (DSA)-negative patients and DSA-positive patients divided according to the class of the DSAs produced (HLA Class I only; HLA Class II only; HLA Class I + II).

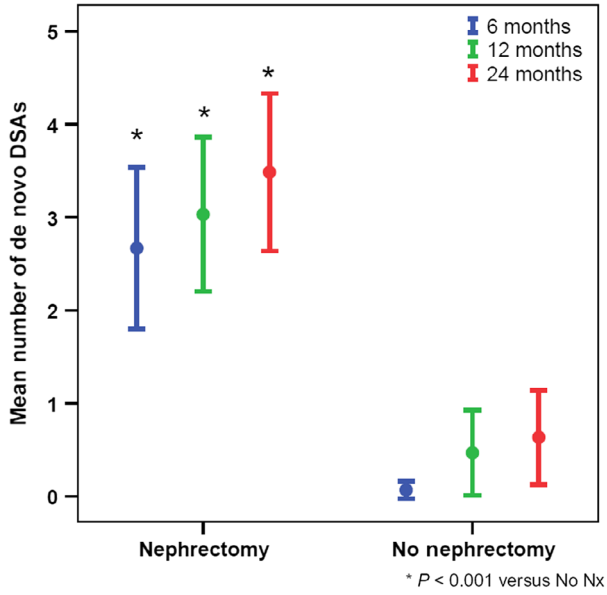

Figure 3 Number of the *de novo* donor-specific antibodies produced in the nephrectomy (NX+) and no-nephrectomy (NX−) group at the 6-, 12-, and 24-month time point. Whiskers represent 95% confidence interval of the mean.

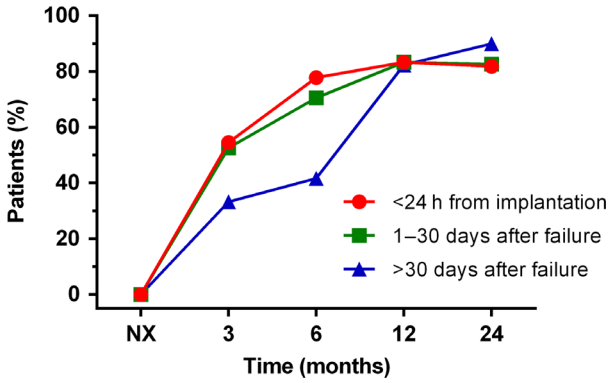

Figure 4 Prevalence of donor specific antibody-positive patients in the nephrectomy group (NX+) according to the timing of nephrectomy.

Tacrolimus withdrawal and HLA antibody production

In the NX+, 50/61 patients had tacrolimus discontinued at the time of the transplant nephrectomy, whereas 11/61 were continued on tacrolimus for 120 ± 90 days. We observed a lower prevalence of patients developing at least one DSA at 3 and 6 months in the subgroup which was continued on tacrolimus after the nephrectomy, although it did not reach statistical significance (22.2% vs. 52.8% at 3 months, $P = 0.100$; 37.5% vs. 70.0% at 6 months, $P = 0.090$). The 3-, 6-, 12-, and 24-month PRA% was

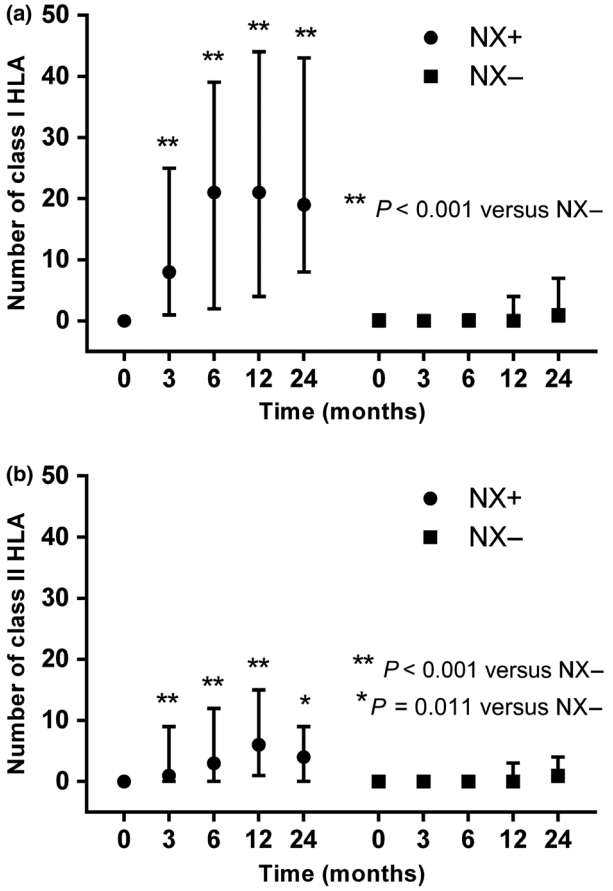

Figure 5 Number of HLA class I (a) and class II (b) antibodies produced in the nephrectomy (NX+) and no-nephrectomy (NX−) group. Whiskers represent interquartile range of the median.

not different in the two subgroups. 45/48 patients in the NX− were continued on tacrolimus after transplant failure, and the levels were available at 24 months in 42. Within this group, DSA-positive patients showed a lower median tacrolimus level compared with DSA-negative [1.9 (0.7–2.6) vs. 4.7 (3.7–6.9) ng/ml, respectively, $P < 0.001$]. ROC analysis revealed that lower tacrolimus levels were associated with DSA production (AUC 0.817, 95% CI 0.633–0.999, $P = 0.002$) with a best cut-off tacrolimus level of 2.9 ng/ml (Youden's index 0.75, sensitivity 0.846, and specificity 0.905). NX− patients with tacrolimus level <3.0 ng/ml were characterized by a higher median cPRA% at 24 months compared with NX− patients with tacrolimus ≥3.0 ng/ml [89 (70–99) vs. 0 (0–43), $P < 0.001$]. Multivariable logistic regression analysis including tacrolimus level, HLA mismatch, history of rejection, previous pregnancy, blood transfusion, showed that a low tacrolimus level was an independent risk factor for DSA formation 24 months after

Table 2. Breadth of HLA antibodies expressed as Panel Reactive Antibody percentage (PRA%).

	Class I PRA%			Class II PRA%			cPRA%		
	NX+	NX−	*P*	NX+	NX−	*P*	NX+	NX−	*P*
NX/failure (*n* = 109)	0 (0–0)	0 (0–2)	N.S.	0 (0–0)	0 (0–0)	N.S.	0 (0–0)	0 (0–19)	N.S.
3 months (*n* = 83)	45 (0—88)	0 (0–0)	<0.001	7 (0–64)	0 (0–0)	<0.001	59 (6–97)	0 (0–0)	<0.001
6 months (*n* = 77)	80 (6–98)	0 (0–1)	<0.001	47 (0–71)	0 (0–0)	<0.001	92 (12–100)	0 (0–4)	<0.001
12 months (*n* = 94)	86 (27–99)	0 (0–25)	<0.001	64 (0–87)	0 (0–44)	<0.001	98 (59–100)	0 (0–64)	<0.001
24 months (*n* = 93)	87 (45–99)	0 (0–40)	<0.001	47 (0–86)	0 (0–66)	0.070	96 (73–100)	38 (0–89)	<0.001
*P**	<0.001	0.072		<0.001	0.002		<0.001	0.001	

cPRA%, cumulative PRA%; N.S., not significant; NX, nephrectomy; NX+, nephrectomy group; NX−, no-nephrectomy group.
Data are expressed as median (interquartile range).
**P* within group variance.

transplant failure (for each 0.1 ng/ml decrease: OR 1.45, 95% CI 1.05–2.00, $P = 0.021$; $R^2 = 0.423$).

Data relating to the long-term continuation of prednisolone were available for 34 NX− patients at the 24-month time point, and we wanted to determine whether tacrolimus had a protective effect on the development of DSAs independently from steroids. We divided the cohort into four groups according to tacrolimus levels (<3 or ≥3 ng/ml, Tac− and Tac+ respectively) and prednisolone (Pred+, on low-dose prednisolone, and Pred−, not on prednisolone), and we found that patients in the Tac+ subgroups showed a lower prevalence of DSA-positive patients regardless of the association with prednisolone (Tac−&Pred−, $n = 9/10$: 90.0%; Tac−&Pred+, $n = 2/3$: 66.7%; Tac+&Pred−, $n = 1/12$: 8.3%; Tac+&Pred+, $n = 1/9$: 11.1%; $P < 0.001$).

Transplant matchability points and grade

The matchability points did not significantly differ between the NX+ and NX− at the time of transplant failure/allograft nephrectomy (4.7 ± 2.5 and 5.3 ± 2.5, respectively, $P = 0.192$) and after 3 months (6.2 ± 2.8 and 5.4 ± 2.5 respectively, $P = 0.221$). Both groups showed a progressive rise in the matchability points with time, which were higher in the NX+ compared with the NX− (7.4 ± 2.8 vs. 5.3 ± 2.5, $P = 0.001$; 7.4 ± 2.9 vs. 5.7 ± 2.6, $P = 0.004$; 7.6 ± 2.8 vs. 6.1 ± 2.7, $P = 0.011$ at the 6-, 12-, 24-month time points, respectively). As a result, the prevalence of patients with "difficult" match was higher in the NX+ compared with the NX−, whereas in the latter we observed a higher prevalence of patients with "moderate" match (Fig. 6). Matchability points did not change over time in NX− patients with tacrolimus levels ≥3.0 ng/ml at 24 months (5.2 ± 2.3 and 5.6 ± 2.3 at the time of graft failure and after 24 months respectively, $P = 0.125$) whereas it did significantly increase within the subgroup of patients with tacrolimus levels <3.0 ng/ml (5.4 ± 2.8 and 6.7 ± 3.0, $P = 0.011$) and was higher compared with the other subgroup ($P = 0.042$).

Discussion

Re-transplantation remains the ideal treatment for patients returning to dialysis after graft failure [2,19]. It is therefore crucial to limit the burden of pretransplant allosensitization which is linked to detrimental graft outcomes and increased mortality [7,8,20], and aim to re-transplant the patient with a favorably matched graft [21]. Transplant nephrectomy has been associated with

Table 3. Risk factors for the development of de novo donor specific antibodies at 12 months.

	Univariable			Multivariable*		
	P	OR	95% CI	*P*	OR	95% CI
Transplant nephrectomy (Y, *n* = 53 vs. N, *n* = 41)	<0.001	15.156	5.514–41.654	0.005	6.915	1.803–26.524
Rejection (Y, *n* = 24 vs. N, *n* = 70)	0.049	2.833	1.005–7.984	0.036	4.707	1.105–20.055
Pregnancy (Y, *n* = 17 vs. male gender, *n* = 59)	0.283	1.891	0.591–6.050	0.541	1.665	0.325–8.530
Transfusions (Y, *n* = 59 vs. N, *n* = 35)	0.002	4.118	1.656–10.238			
Transfusion events at/after Nx/Tx failure	0.001	1.439	1.172–1.767	0.490	1.100	0.839–1.443
Total number of HLA mismatches (A/B/Cw/DR/DQ)	0.121	1.155	0.963–1.386	0.099	1.268	0.956–1.682
Transplant duration (months)	<0.001	0.964	0.948–0.980	0.033	0.977	0.957–0.998

N, no; Nx, nephrectomy; Tx, transplant; Y, yes.

*R^2 = 0.567.

Table 4. Risk factors for the development of de novo donor specific antibodies at 24 months.

	Univariable			Multivariable*		
	P	OR	95% CI	*P*	OR	95% CI
Transplant nephrectomy (Y, *n* = 45 vs. N, *n* = 48)	<0.001	8.266	3.086–22.140	0.008	6.118	1.564–23.928
Rejection (Y, *n* = 27 vs. N, *n* = 66)	0.059	2.860	0.962–8.501	0.038	4.025	1.082–14.973
Pregnancy (Y, *n* = 20 vs. male gender, *n* = 58)	0.435	1.579	0.501–4.976	0.734	1.286	0.301–5.500
Transfusions (Y, *n* = 71 vs. N, *n* = 22)	0.005	4.253	1.558–4.253			
Transfusion events at/after Nx/Tx failure	0.007	1.293	1.071–1.560	0.597	1.074	0.825–1.397
Total number of HLA mismatches (A/B/Cw/DR/DQ)	0.056	1.226	0.995–1.512	0.049	1.316	1.001–1.741
Transplant duration (months)	0.001	0.973	0.958–0.989	0.421	0.992	0.972–1.012

N, no; Nx, nephrectomy; Tx, transplant; Y, yes.

*R^2 = 0.408.

the early development of HLA antibodies, which variably persist in time [9,10,22]. Against this background, our study shows that transplant nephrectomy: (i) leads to the long-term production of donor and nondonor specific HLA antibodies which significantly rises over time, compared with patients not undergoing transplant nephrectomy after graft failure and even if the nephrectomy was performed few hours after the engraftment; (ii) has a negative impact on the likelihood of the patient finding a second HLA-compatible transplant; (iii) is a significant predictor of the mid- (12 months) and long-term (24 months) formation of *de novo* DSAs. Importantly, our data show that in patients with an allograft *in situ*, the maintenance of tacrolimus with trough levels ≥3 ng/ml is protective against the long-term production of donor and nondonor specific HLA antibodies and is associated with a favorable re-transplantation matchability up to 2 years after the failure of the first graft. In the NX− cohort, we also noticed a more pronounced class II-oriented immune response. There is no clear explanation for this, although it has been hypothesized that since class I antigens are constitutively expressed on the donor kidney [12], DSAs against them might be retained by the kidney graft [23].

The benefits and safety of maintaining immunosuppression in preventing allosensitization after transplant failure has been questioned in several reports, although the choice of immunosuppression withdrawal remains physician-dependent rather than protocol driven [24,25]. Woodside and colleagues reported the link between immunosuppression and hospitalization for infective diseases in patients with failed graft. On the other hand, they also reported a higher rate of noninfection related hospitalization for febrile illness in patients with failed graft *in situ* and weaned off the immunosuppression, which led to allograft nephrectomy in most of the cases [26]. This may suggest the protective effect of immunosuppression against the development of the inflammatory state typical of the chronically rejected retained allograft [27]. Immunosuppression withdrawal has been associated with the risk of becoming highly sensitized after transplant failure in multivariable analysis models [11,28]. In our

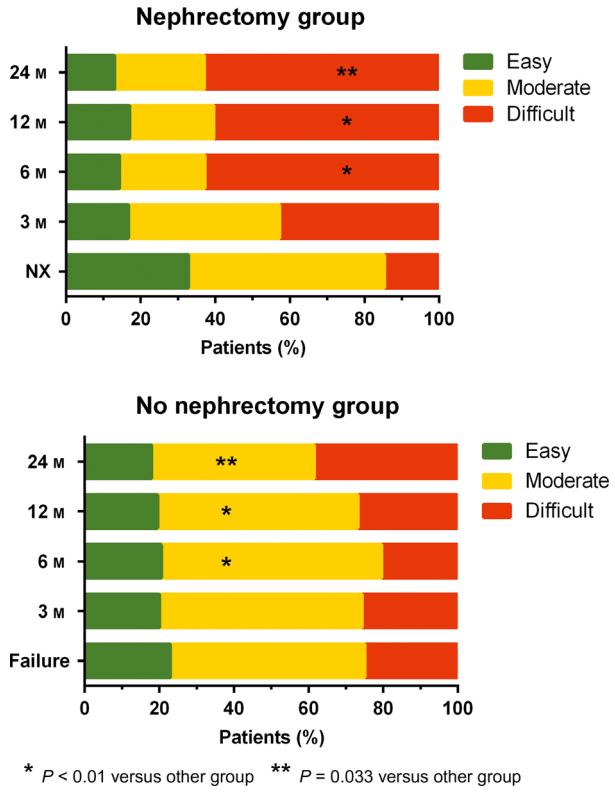

Figure 6 Changes of the matchability grade with time in the nephrectomy (NX+) and no-nephrectomy (NX−) group.

NX− group, both donor and nondonor HLA antibody production and a difficult transplant matchability score were significantly correlated with low tacrolimus levels. This is the first report showing the potential benefits of maintaining tacrolimus levels above a specific threshold – namely ≥3.0 ng/ml – to reduce the risk of allosensitization and increase the chances of re-transplantation in patients not undergoing nephrectomy. However, in patients undergoing nephrectomy, our data are suggestive of a limited protective effect of tacrolimus restricted to the first 3–6 months after nephrectomy, during which a subgroup of the cohort was briefly continued on immunosuppression.

In the United States, about 20% of wait-listed dialysis patients undergo transplant nephrectomy [29]. Although two recent meta-analyses have suggested that allograft nephrectomy could be linked to worse second allograft outcomes [30] and longer time on the waiting list [14], the impact on the likelihood of finding a HLA-compatible transplant has not been hitherto investigated. Deceased-donor organ allocation policies take into account a number of clinically relevant factors, of which blood group, HLA type and allosensitization are the most important. In our study, given the relative homogeneity of the two in-study groups (first transplant, no difference in the ethnicity, blood group distribution and cPRA% at the time of transplant failure/transplant nephrectomy), we used the matchability grade as a surrogate estimate of the chances of re-transplantation. We found that the likelihood of finding a HLA-compatible kidney was lower in the NX+ compared with the NX− as soon as 6 months after nephrectomy, becoming significantly worse with time.

Important strengths of our study are: (i) the selection of first-graft recipients with no detectable DSAs at the time of graft failure or transplant removal, and (ii) the study duration up to 24 months. The significance of this relates to the fact that previous reports have included patients who were DSA positive before the nephrectomy and patients with multiple renal transplants. These are additional variables shown to be associated with DSA development independent of nephrectomy or graft failure [7]. Finally, the study time extended to 24 months allowed to capture a significant increase with time of the allosensitization also in the control group, which has important implications on re-grafting and therefore on the decision of continuing the immunosuppression in patients who are candidates for re-transplantation, especially if no living donor options are available.

Our study has several limitations. This is a retrospective study carried out over several years, and because of this, we have not been able to collect hospital admissions during the study time. Furthermore, the matchability score only takes immunological factors into account and does not include important determinants such as age and comorbidities. The relatively small number of patients continued on tacrolimus after nephrectomy because of a different calcineurin-inhibitor management protocol in our Unit before 2011 and the short duration of treatment might have affected our observations and does not allow to make definitive statements regarding the benefits of continuing tacrolimus after transplantectomy. DSA development might be underestimated by the use of low-resolution typing (e.g., missed because the donor's HLA molecules are not represented in the assay reagents) or in other situations DSA could be overestimated (e.g., the donor is DR4 and DR4 antibodies are determined to be DSA because one or more DR4s in the reagents are positive, but the donor's DR4 molecule is negative). Finally, the antibody assay is designed to be saturating and whilst reports are based upon the strength of the MFI level, they should be considered as semi-quantitative. The discussion and inferences derived from this study related

to MFI levels should be viewed in light of this limitation.

This study shows that graft failure is associated with increasing allosensitization, which occurs even in patients who have had nephrectomy within 24 h of implantation. Patients who undergo nephrectomy tend to produce more antibodies than those who do not, and this is reflected by the rising cPRA with time which negatively impacts on the chances of finding a second HLA-compatible transplant. It is not clear if this could be mitigated by tacrolimus in patients undergoing nephrectomy, whereas our data suggest that tacrolimus levels ≥3 ng/ml may reduce antibody production in patients who do not undergo nephrectomy. Significant numbers of patients who have had a failed graft remain on waiting lists for long periods of time. This is becoming a significant problem, especially considering that patients experiencing early graft loss for vascular complications are most often medically fit for re-transplantation, and at present there are no specific treatments or protocols for their management. There is therefore an urgent need for further studies and particularly, whether tacrolimus continuation or other immunosuppressive agents after graft loss have any beneficial effect [31].

Taken together, our findings suggest that the maintenance *in situ* of a failed kidney graft and the continuation of low dose tacrolimus prevent the development of allosensitization and can potentially increase the chances of re-transplanting a HLA-compatible kidney. In light of our results showing a significant alloimmune response after graft failure in both NX+ and NX− by 3 and 12 months, respectively, the avoidance of non-clinically indicated nephrectomy should be sensibly considered in candidates for re-transplantation, especially those who received a less favorably HLA-mismatched graft or had rejection in the past. Tacrolimus levels should be carefully monitored in patients not undergoing allograft nephrectomy and our data suggest that a trough level ≥3 ng/ml should be maintained. Consideration should be given to the timing of relisting and re-transplantation, as early re-transplantation may avoid the later development of HLA antibodies. Larger, multicenter prospective studies are urgently required.

Authorship

G.L., D.T.: study design; GL: data collection, statistical analysis, manuscript preparation; P.B., E.S.: generating single-antigen data and manuscript review; N.F., N.G.: laboratory data generation; S.H, A.W.: data collection; P.H., V.P., M.W., D.T.: manuscript review.

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Conflicts of interest

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