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Risk factors for development of panel reactive antibodies and their impact on kidney transplantation outcome

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Abstract. The impact of potential risk factors for development of panel reactive antibodies (PRA) in 1078 cadaveric kidney graft recipients was investigated in a multivariate analysis. Multiple transplantation, transfusion of more than five blood units and more than two pregnancies were revealed as factors with a significant independent impact on the formation of high levels of PRA. Multiple transplantation and polytransfusion also affected primary non-function, initial function and long-term graft survival at 1, 3 and 5 years. Incidence of early rejection (within 30 days) was significantly increased with repeated transplantation and decreased with a full-house HLA match. However, these effects on transplantation outcome could only be observed when risk factors lead to the formation of antibodies. In patients with risk factors present, but without subsequent sensitization, the graft survival expectation was the same as in patients in whom risk factors were absent.

Key words: Panel reactive antibodies – Risk factors – Kidney transplantation – Transplantation outcome

High levels of panel reactive antibodies (PRA) are known to be a risk factor for the outcome of renal transplantation. Although some authors have shown that good results can be achieved in patients with elevated PRA [3], and that successful transplantation is possible across the barrier of a positive crossmatch [9], many centres still yield less satisfactory results in sensitized patients [1, 8, 10]. There have been attempts to improve the chances for this group of patients through international exchange programmes which allow the priority transplantation of these patients or even grafting of organs with acceptable HLA compatibility [3, 5]. We investigated which of the factors suspected to have an impact on the development of PRA would prove significant and what their impact was on transplantation outcome. In a subset analysis the independent impact of risk factors on transplantation outcome was investigated.

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Patients and methods

The analysis was carried out on the cohort of cadaveric transplant recipients at our transplant unit in the cyclosporine era. All patients between 1982 and 1991 with available data on preoperative course (duration of kidney disease, waiting time on dialysis, number of blood units transfused prior to transplantation and number of pregnancies) and postoperative follow-up (eventual rejection episodes during the first 30 days, initial function and long-term follow-up) were entered into the study. Grafts lost immediately for technical reasons were excluded ($n = 16$, 1.3%) with these restrictions, out of a total of 1222 transplants performed, 1078 were able to be analysed.

The cohort was divided into four groups according to PRA level. Group one (GR1) comprised 762 patients with no antibodies, group two (GR2) 142 patients with 1–20% PRA (low sensitization), group three (GR3) 105 patients with 21–60% PRA (intermediate sensitization) and group four (GR4) 69 patients with 61–100% PRA (highly sensitized). The following potential risk factors were investigated:

1. Multiple transplantation (MT) (first, $n = 895$; second, $n = 146$; third or subsequent, ($n = 37$)
2. Blood transfusions (BT) (none, $n = 149$; 1–5, $n = 474$; 5–10, $n = 181$; 10 or more, $n = 274$)
3. Pregnancies (PR) (none, $n = 140$; 1–2, $n = 175$; 3 or more, $n = 104$)
4. Duration of kidney disease (DD) (0–5 years, $n = 510$; over 5 years, $n = 568$)
5. Recipient sex (female, $n = 419$; male, $n = 659$)
6. Recipient age (1–77 years, mean 43.4 ± 14.1 years continuously)

All interactions of the above factors were also investigated. Risk of sensitization was analysed by stepwise polychotomous logistic regression. Factors that revealed a significant impact on the development of PRA were analysed as risk factors for transplantation outcome by their influence on primary non-function (never functioning transplants) (PNF), inital function (IF) (measured by urine output in the first 24 h) [0–200 ml (anuria), 201–1500 ml (oliguria), > 1500 ml (normal diuresis)], incidence of rejection episodes in the first 30 days (ERE) and long-term function (LTF) (at 1, 3 and 5 years). Methods used were the Chi-squared test, Kaplan Meier estimates, stepwise logistic regression and Cox's multivariate proportional hazards model analysis where appropriate. The results of Kaplan Meier estimates are given as percentage of functioning grafts, with standard error (probabilities are given for Breslow and Mantel-Cox tests). The results from stepwise logistic regression and Cox's hazards model analysis are given in relative risks (RR) which indicate the increase in the probability of occurrence of the predicted event.

Transplantation was carried out only after a negative preoperative T-cell cross-match with recent recipient serum (not older than 3 months). The technical details of transplantation and the mode of immunosuppression are described elsewhere [7].

Table 1. Relative risk (RR) and 95% confidence interval (CI 95%) of independent risk factors for the development of panel reactive antibodies (PRA). MT, multiple transplantation; BT, blood transfusions; PR, pregnancies

	PRA						
	0%	1–20%		21–60%		>60%	
	RR	RR	CI 95%	RR	CI 95%	RR	CI 95%
MT							
2nd	1.0	2.2	1.3–3.7	2.2	1.3–4.0	5.7	3.0–11.0
≥3rd	1.0	2.0	0.51–7.9	10.0	3.7–29.0	43.0	16–120.0
							$P < 0.0001$
BT							
1–5	1.0	1.5	0.79–2.8	2.4	0.98–6.1	0.88	0.32–2.4
6–10	1.0	1.5	0.72–3.1	3.9	1.5–10.0	2.1	0.77–5.8
≥11	1.0	3.2	1.7–6.2	5.2	2.1–13.0	3.7	1.5–8.9
							$P < 0.0001$
PR							
1–2	1.0	1.1	0.7–1.9	1.9	1.1–3.3	2.1	0.98–4.3
>2	1.0	1.2	0.63–2.3	2.9	1.5–5.5	6.4	2.6–14.0
							$P < 0.0001$
Recipient age							
1 year	1.0	1.0	0.99–1.0	0.99	0.97–1.0	0.98	0.95–1.0
							$P = 0.070$

Results

Risk factors for PRA formation

Factors with an independent impact on development of PRA were MT with a RR after the second graft of 2.2 for GR2, 2.2 for GR3 and 5.7 for GR4, and after the third graft 2.0 for GR2, 10 for GR3 and 43 for GR4. BT showed a RR of 1.5 (GR2), 2.4 (GR3) and 0.88 (GR4) for 1–5 BT, 1.5 (GR2), 3.9 (GR3) and 2.1 (GR4) for 6–10 BT and 3.2 (GR2), 5.2 (GR3) and 3.7 (GR4) for >10 BT. PR caused an increase in RR of 1.1 (GR2), 1.9 (GR3) and 2.1 (GR4) for 1–2 PR and 1.2 (GR2), 2.9 (GR3) and 6.4 (GR4) for >2 PR. Recipient age showed a RR of 1 (GR1), 0.99 (GR2) and 0.98 (GR3) for each 1-year step. Significance levels were $P < 0.0001$ for MT, BT and PR, and $P < 0.07$ for recipient age (Table 1).

In the analysis of interactions among the single variables, none of the interactions revealed an independent impact on development of PRA.

Impact of different PRA levels on waiting time and outcome parameters

Waiting time for transplantation. Waiting time for GR1 was 24.9 months, GR2 31.7 months, GR3 36.1 months and GR4 39.8 months ($P = 0.007$ for GR1 vs. GR2, $P < 0.0001$ for GR1 vs GR3, GR4). The differences between the other groups did not reach significance.

Primary non function. Incidence of PNF (overall 72 patients) was 42 patients (5.5%) in GR1, nine (6.3%) in GR2, nine (8.6%) in GR3 and 12 (17.4%) in GR4 ($P = 0.002$).

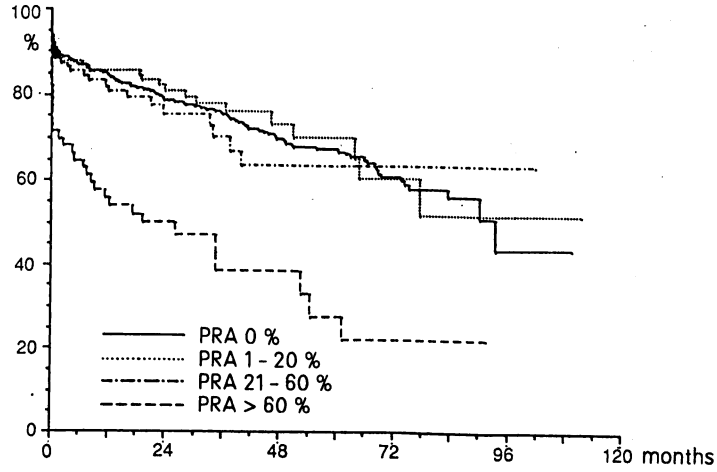

Fig. 1. Percent graft survival for different PRA levels (Kaplan Meier estimates, $P < 0.0001$ Breslow, $P < 0.0001$ Mantel-Cox GR1, 2, 3 vs. GR4)

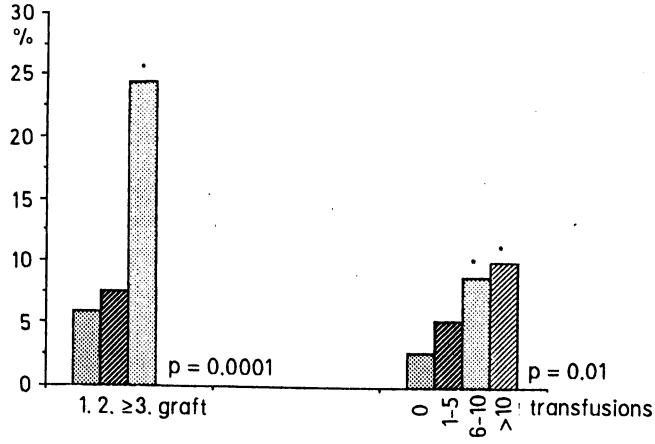

Fig. 2. Differences in incidence of PNF for number of transplantations and number of transfusions

Initial function. IF was anuria for 135 patients (17.7%) in GR1, 35 (24.6%) in GR2, 21 (20%) in GR3 and 20 (29%) in GR4. Grafts showed oliguria in 149 patients (19.5%) in GR1, 14 (9.9%) in GR2, 25 (23.8%) in GR3, and 16 (23.2%) in GR4. Normal diuresis was established in 478 patients (62.8%) in GR1, 93 (65.5%) in GR2, 59 (56.2%) in GR3, and 33 (47.8%) in GR4) ($P = 0.037$).

Early rejection episodes. Total incidence was 518 (48%). Of these, 428 patients (39%) had single episodes (SRE) and 90 (8%) multiple (MRE). SRE occurred in 41.7% in GR1, 37.4% in GR2, 30.7% in GR3 and in 55.7% in GR4. Incidence of MRE within the first 30 days was 6.5% in GR1, 6.1% in GR2, 7.9% in GR3 and 14.8% in GR4 ($P = 0.001$; GR1, 2 and 3 vs GR4).

Long-term function. LTF at 1, 3 and 5 years was $84.5 \pm 1\%$, $75.5 \pm 2\%$ and $67.4 \pm 2\%$ for GR1; $85.3 \pm 3\%$, $77.6 \pm 4\%$ and $69.9 \pm 5\%$ for GR2; $81.8 \pm 4\%$, $69.8 \pm 6\%$ and $63.4 \pm 7\%$ for GR3; and $55.6 \pm 6\%$, $38.4 \pm 7\%$ and $27.4 \pm 8\%$ for GR4 ($P < 0001$; GR1, 2 and 3 vs GR4) (Fig. 1).

Impact of risk factors on outcome parameters

Incidence of PNF.
Multiple transplantation: one graft, $n = 52$ (5.8%); two grafts, $n = 11$ (7.5%); three or more grafts, $n = 9$ (24.3%) ($P < 0.0001$).

Blood transfusions: none, $n = 4$ (2.7%); 1–5 units, $n = 25$ (5.3%); 5–10 units, $n = 16$ (8.8%); >10 units, $n = 27$ (9.8%) ($P = 0.01$).

Pregnancies: none, $n = 53$ (4.9%); one or two, $n = 13$ (7.4%); more than two, $n = 6$ (5.7%) (n.s.).

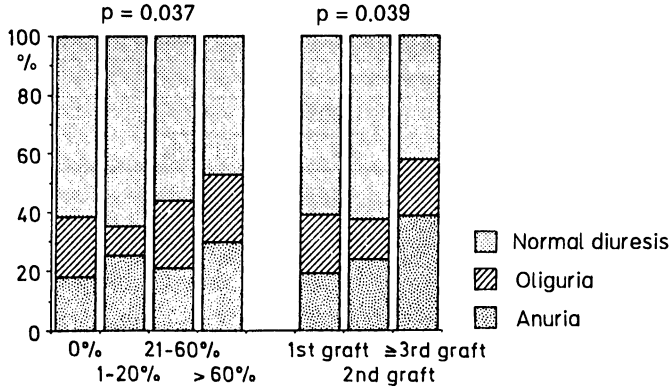

Fig. 3. Differences in initial function (first 24 h) for PRA levels and number of transplantations

Duration of kidney disease: <5 years, $n = 33$ (6.5%); ≥5 years, $n = 39$ (6.9%) (n.s.) (Fig. 2).

Initial function. Multiple transplantation: For first grafts, anuria in 163 patients (18.2%), oliguria in 177 (19.8%) and normal diuresis in 555 (62%); for second grafts, anuria in 34 patients (23.3%), oliguria in 20 (13.7%) and normal diuresis in 92 (63%). For third or subsequent grafts, anuria in 14 patients (37.8%), oliguria in 17 (18.9%) and normal diuresis in 16 (43.3%) ($P = 0.039$ (Fig. 3).

Blood transfusions, pregnancies and duration of kidney disease did not significantly affect initial function.

Incidence of rejection episodes. In addition to the suspected risk factors for PRA development, HLA A, B and DR mismatch were included in the stepwise logistic regression analysis for the occurrence of rejection. The following variables revealed a significant independent impact on the occurrence of single (SRE) or multiple (MRE)

Table 2. Relative risk (RR) and 95% confidence interval (CI 95%) of independent risk factors for the occurrence of early rejection episodes (ERE) [single (SRE) or multiple (MRE)] in all patients and in non-sensitized patients only. MT, multiple transplantation; FH, full-house HLA match

	ERE				
	0	1 (SRE)		>1 (MRE)	
	RR	RR	CI 95%	RR	CI 95%
Sensitized patients					
MT					
2nd	1.0	1.2	0.81–1.8	1.5	0.74–3.0
≥3rd	1.0	6.4	2.2–19.0	14.0	4.1–51.0
					$P < 0.0001$
Non-sensitized patients					
MT					
2nd	1.0	not in the model		not in the model	
≥3rd	1.0	not in the model		not in the model	
Sensitized patients					
FH					
yes	1.0	0.40	0.21–0.77	0.59	0.18–2.0
					$P = 0.012$
Non-sensitized patients					
FH					
yes	1.0	0.38	0.19–0.76	0.68	0.20–2.3
					$P = 0.015$

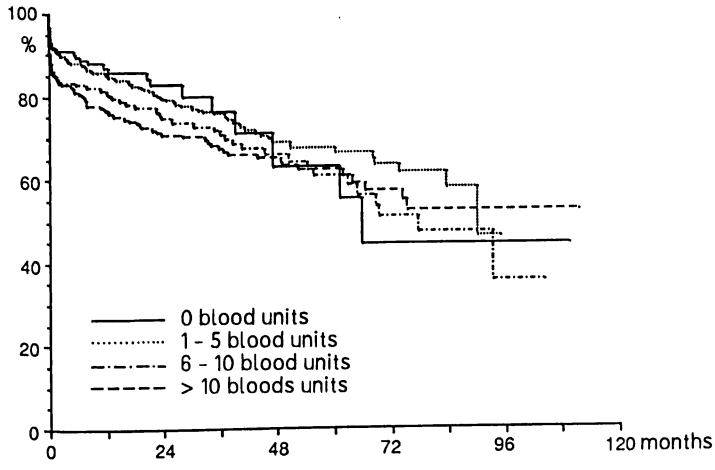

Fig. 4. Percent graft survival by number of blood units transfused (Kaplan Meier estimates, $P < 0.015$ Breslow, $P < 0.002$ Mantel-Cox)

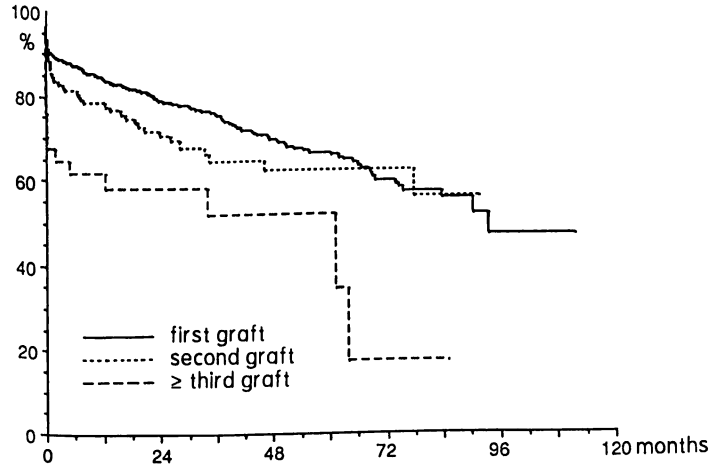

Fig. 5. Percent graft survival by number of transplantations (Kaplan Meier estimates, $P = 0.001$ Breslow, $P < 0.0001$ Mantel-Cox GR1, 2, 3 vs GR4)

rejection episodes. MT showed a RR for SRE of 1.2 for second transplantations and 6.4 for third or subsequent transplantations. The RR for development of MRE was 1.5 for second transplantations and 14 for third or subsequent transplantations ($P < 0.0001$). A six-loci HLA match significantly reduced the risk of SRE and MRE. RR for full-house (FH) match was 0.40 for SRE and 0.59 for MRE ($P = 0.012$).

In the cohort of non-sensitized patients (GR1), the only significant independent risk factor for the occurrence of ERE was a FH match with a RR of 0.38 for SRE and 0.68 for MRE ($P = 0.015$) (Table 2).

The effects of risk factors on LTF were analysed by univariate Kaplan-Meier estimates and Cox's multi-variate proportional hazardous model. Factors having significant impact on LTF in the univariate analysis were BT and MT. Patients who had received no BT had a 1-, 3- and 5-year graft survival of 86.9 ± 2%, 75.5 ± 5% and 63.7 ± 9%. The graft-function rates for one to five BT were 85.5 ± 1%, 76.3 ± 2% and 68.7 ± 2%, and with five to ten BT the rates were 80.5 ± 2%, 69.8 ± 3% and 60.8 ± 4%. For more than ten BT the graft survival was 76.1 ± 2%, 67.3 ± 3% and 61.4 ± 3% ($P = 0.002$ Breslow; $P = 0.015$ Mantel) (Fig. 4).

MT affected graft function at 1, 3 and 5 years as follows: first graft had function rates of 84.5 ± 1%, 75.4 ± 1% and 66.5 ± 2%, respectively; second grafts 77 ± 3%, 63.6 ± 4% and 61.6 ± 5%, respectively; and third, fourth and fifth grafts had function rates of 61.1 ± 7% at 1 year and 52.1 ± 8% at 3 and 5 years ($P = 0.001$ Breslow; $P < 0.0001$ Mantel) (Fig. 5).

When calculations were carried out for non-sensitized patients only (GR1) no statistically significant effect of the risk factors on graft survival could be demonstrated.

In the multivariate proportional hazards model, in addition to the suspected factors for development of PRA, variables considered as potentially affecting graft survival were entered: donor and recipient age, donor and recipient sex, A, B and DR match, warm and cold ischaemic time and occurrence of early rejection.

Factors increasing the risk of grafts loss were MT [RR 1.37 for second grafts and 1.88 for third and subsequent grafts ($P = 0.008$)], first warm ischaemic time [≤ 5 min, RR 1.3; > 5 min, RR 1.75 ($P = 0.031$)] and incidence of early rejection [RR 1.35 ($P = 0.014$)]. Factors decreasing

Table 3. Relative risk (RR) for graft loss. Variables (RF) with significant influence. Analysis for all patients and for non-sensitized patients only

	RR	*P*-value
Multiple transplantation:		
PRA > 0		
2nd	1.3	
≥ 3rd	1.8	0.008
PRA = 0		
2nd		
≥ 3rd		not in the model
Warm ischaemic time:		
PRA > 0		
1–5	1.3	
6–10	1.7	0.031
PRA = 0		
1–5	1.4	
6–10	2.1	0.031
Early rejection episode:		
PRA > 0		
1	1.3	
> 1	1.5	0.014
PRA = 0		
1		
> 1		not in the model
Recipient age:		
PRA > 0		
1 year	0.98	
10 years	0.82	< 0.0001
PRA = 0		
1 year	0.97	< 0.0001
Disease duration:		
PRA > 0		
> 5 years	0.74	0.024
PRA = 0		
> 5 years	0.70	0.011

the risk of graft loss were longer duration of kidney disease [> 5 years, RR 0.74 ($P = 0.024$)] and increasing recipient age [1 year age difference, RR 0.98, and 10 years age difference, RR 0.82 ($P < 0.0001$)].

When calculations were carried out for non-sensitized patients only (GR1) no independent statistically significant effect on graft survival could be demonstrated for the risk factors for PRA formation. The factors remaining significant were warm ischaemic time [1–5 min, RR 1.4 > 5 min, RR 2.1 ($P = 0.006$)], recipient age 1 year age difference, [RR 0.97 ($P < 0.0001$)] and duration of kidney disease ≥ 5 years, RR 0.70 ($P = 0.011$)] (Table 3).

Discussion

Almost all of the suspected risk factors seem to affect the development of PRA in a rather complex way. The relative risk for low sensitization (GR1) is hardly influenced by BT and PR as indicated by low RR and confidence intervals. The correlation of few BTs and few PRs with the formation of low PRA levels seems to be loose. Few BTs (0–5) also decreased the risk of high sensitization, a finding that can be partly seen in terms of the transfusion effect [6]. However polytransfusion, multiple pregnancies and multiple transplantation increased the RR for PRA development significantly. MT in particular showed an impressive effect on sensitization. Patients with a history of more than one prior graft have a 40 times higher risk of high sensitization in comparison with first graft recipients.

The impact of increasing recipient age, which lowered the risk for sensitization, was small for the single-year step but decreased the RR by 30% for a recipient age difference of 10 years. This finding is in accordance with the phenomenon of a better transplantation outcome in older recipients because of a decreased immunological response in these individuals [11].

In the presence of PRA, independent factors for single and multiple ERE were MT, which had a strong detrimental effect, and FH HLA match, which reduced the RR of rejection by half. The occurrence of early rejection was not increased by MT in non-sensitized patients. In this cohort only a six-loci HLA match reduced the risk of early rejection significantly. A match of less than six HLA loci did not decrease the risk of early rejection episodes in our cohort of patients.

Early transplantation outcome was affected by multiple transplantations and multiple blood transfusions. These variables showed a detrimental effect in univariate and multivariate analysis. Of all other factors that were entered into the Cox model for graft survival, only increasing warm ischaemic time was found to increase the RR of graft loss, while increasing recipient age and increasing duration of kidney disease decreased the RR of graft failure. The detrimental effect of long-term kidney disease may be an explanation for the finding that a long history of kidney disease has a beneficial impact on graft survival.

HLA matching did not appear to be an isolated factor among the variables entered, which is in contrast to previously published results from multicentre analysis [4]. This finding, documented in a homogeneous cohort of over 1000 grafts followed up at a single centre suggests that the possible benefits of HLA matching are completely masked by stronger effects of several other risk factors.

However, the effects of PRA risk factors on transplantation outcome only show relevance when PRA have been formed. In recipients who have not developed PRA despite risk factors present, MT, BT and PR do not have isolated effects on initial diuresis nor effects on the incidence of PNF or LTF. Here the univariate Kaplan Meier estimates show no significant diversion of the graft function curves. In the Cox model for graft survival in non-sensitized patients, only recipient age, warm ischaemic time and duration of kidney disease revealed an independent influence. PRA act as the mediator and sole effector of risk factor influence on transplantation outcome.

Despite graft exchange programmes for sensitized patients, preoperative crossmatching and aiming for a favourable HLA match, results of transplantation in patients with high PRA levels are still poor. Highly sensitized patients often enter a vicious circle of long waiting times, early graft loss and retransplantation which again increases the risk of further formation of PRA. On the other hand, there seems to be a certain chance of not being sensitized by the risk factors described which gives recipients with risk factors present, but without the subsequent formation of PRA, the same expectation of graft survival as non-sensitized patients.

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