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# HLA-DRB1 compatibility in cadaver kidney transplantation: correlation with graft survival and function

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**Abstract** The introduction of genomic HLA-DR typing has stimulated a re-evaluation of the role of HLA-DR compatibility on cadaver kidney transplantation. We retrospectively studied the influence of HLA-DRB1 matching on the survival of 416 patients using univariate and Cox regression analysis as well as its influence on the occurrence of rejection episodes and on creatinine level at the 3rd month in the 198 recipients for whom these data were available. The following parameters were also considered: HLA-A,B compatibility, donor and recipient age, graft number, pre-transplant blood transfusions and panel reactive antibodies (PRA). Twenty-four month graft survival was 100 % for transplants with zero mismatches ($n$ = 47), 87.9 % for those with one mismatch ($n$ = 191) and 81.3 % for those with two mismatches ($n$ = 178). In the Cox model, HLA-DRB1 matching was the most significant variable influencing graft survival (47 % of $\chi^2$ $P$ = 0.001), followed by HLA-A,B matching (23 %, $P$ = 0.02) and donor age (19 %, $P$ = 0.04). Ninety-two percent of the patients with zero mismatches experienced no rejection episodes in the first 3 post-transplant months compared with 62 % and 41 % of patients with one and two mismatches, respectively. Mean creatinine level (mg/dl) was 1.2, 1.4, and 1.5 in patients with zero, one, and two mismatches, respectively. Should these results be confirmed by prospective studies, HLA-DRB1 compatibility will have to be considered as an organ allocation criterion.

**Key words** HLA-DRB1, kidney transplantation · Kidney transplantation, HLA-DRB1 Graft survival, HLA-DRB1, kidney

## Introduction

The role of HLA-DR compatibility on the outcome of cadaveric kidney transplants has been a matter of debate up until recently [9, 15]. The consistent error rate associated with HLA-DR serological techniques might be responsible for the lack of correlation between HLA-DR matching and graft outcome reported by some centers [2, 4, 6, 20].

HLA-DR typing at the DNA level has been shown to be more accurate than serological typing [10, 11, 17, 22], and is practiced routinely today by many laboratories.

Thus far, few studies on the role of HLA-DRB1 compatibility on graft survival and function have been published [1, 5, 7, 13, 19], and for the latter in particular, the data involve only a limited number of cases [1, 7]. The aim of the present study was to retrospectively investigate, in our setting, the effect of HLA-DRB1 generic matching on graft survival and on early kidney function.

**Table 1** Variables of patients studied according to HLA-DRB1 mismatches

| Variables | Number ot HLA-DRB1 mismatches | | |
|---|---|---|---|
| | 0 ($n = 47$) | 1 ($n = 191$) | 2 ($n = 178$) |
| Gender: female/male | 15/32 | 70/121 | 56/122 |
| Recipient age (years) Median (10–90 percentiles) | 40 (19–57) | 43 (23–56) | 40.5 (20–53) |
| Donor age (years) Median (10–90 percentiles) | 26 (15–50) | 30 (17–56) | 31.5 (16–54) |
| First grafts | 44 (93.6) | 177 (92.7) | 167 (93.8) |
| Nonsensitized recipients | 39 (83.0) | 165 (86.4) | 148 (83.2) |
| Transfused recipients | 24 (51.1) | 101 (52.9) | 79 (44.4) |
| HLA-A,B 0–2 mismatches | 37 (78.7) | 155 (81.2) | 133 (74.7) |
| 3–4 mismatches | 10 (21.3) | 36 (18.8) | 45 (25.3) |

## Materials and methods

### Study design, organ allocation, patients

Since 1989 we have collected blood samples from donor/recipient pairs at transplantation in order to perform some retrospective immunological tests. In the present study, DNA samples were available for 416 out of 670 nondiabetic subjects transplanted from 1 January 1989 to 24 April 1993 in 5 of the 12 centers that take part in the North Italy Transplant program (NITp) [16]. Two transplant centers also provided data on early kidney function for all of their 198 patients. Upon registering for the waiting list, all patients were informed that their blood samples would be used for histocompatibility testing. The participating centers shared the same tissue typing laboratory and similar protocols for patient management.

In the NITp, kidney allocation criteria include ABO identity, HLA-A,B (but not HLA-DR) compatibility, and a negative pretransplant crossmatch between total donor lymphocytes and recipient current and historical sera [21].

### Immunosuppressive treatment and rejection diagnosis

Patients studied were treated with cyclosporin and steroids, with or without azathioprine. A diagnosis of rejection was made using clinical parameters (25 % reduction in renal function, graft tenderness, fever, proteinuria) and occasionally by fine needle aspiration or core renal biopsy. Rejection episodes were treated with intravenous methylprednisolone pulses.

### Graft function evaluation

Kidney function was assessed with the following parameters:
1. Serum creatinine level (mg/dl) at the 3rd post-transplant month
2. Occurrence of clinically evident rejection episodes (yes/no) during the first 3 months following transplantation
3. Number of steroid pulses during the first 3 post-transplant months

### DNA typing

HLA-DRB1 generic typing was performed using sequence-specific oligonucleotide (SSO) typing on polymerase chain reaction (PCR)-amplified DNA. The SSO probes used allowed typing of HLA-DRB1 *01–14 specificities [3].

### Statistical analysis

Graft survival rates were calculated with the actuarial method. Patient death or return to chronic replacement treatment was considered a failure. Ninety-five percent confidence intervals (CI) were calculated for 12- and 24-month survival estimates.

The Cox proportional hazard regression model was applied to study the influence on graft survival of the following variables: HLA-DRB1 mismatches (0, 1, or 2), HLA-A,B mismatches (0–2, 3–4), donor age (0–50 years, > 50 years), recipient age (0–55 years, > 55 years), previous transplants (first graft, regrafts), PRA (0, 1 %–100 %), and number of pretransplant blood transfusions (0, > 0). Multivariate analysis was performed three times including transplant centers as covariates, stratifying for transplant center and, lastly, not considering transplant center.

Analysis was performed according to the following steps:
1. Individual test score for all the covariates (chi-square score)
2. Forward stepwise sequence of chi-square statistics in the order of greatest increase to the overall test statistic
3. Inclusion in the final model of the covariates with significant ($P < 0.05$) chi-square increment

Regression coefficients were also expressed as relative risk (RR) with 95 % CI.

As for graft function, according to the different HLA-DRB1 mismatch groups, the following analyses were carried out:
1 Proportion and 95 % CI of patients with no rejection episodes
2. Median and 10–90 percentiles of the total number of steroid pulses administered to patients who experienced at least one rejection episode
3. Mean and 95 % CI of reciprocal creatinine value at the 3rd post-transplant month

Data were analyzed using the Statistical Analysis System (SAS Institute, Cary, N. C., USA).

## Results

Table 1 reports the characteristics of patients according to zero, one, or two HLA-DRB1 mismatches; it shows that patients were rather uniformly distributed for the variables considered.

Table 2 shows actuarial graft survival analysis for the single variables. While no significant effect on survival was found for recipient age, PRA, pretransplant blood transfusions, or graft number, kidney survival was influenced by HLA matching (both HLA-DRB1 and HLA-A,B) and donor age. In particular, transplants with zero HLA-DRB1 mismatches had a 19 % higher survival rate at 24 months than grafts with two mismatches; a 10 % difference in graft survival was evidenced between transplants with 0–2 and 3–4 HLA-A,B mismatches and between transplants performed with kidneys from donors below 50 years and those 50 years of age or older.

**Table 2** Actuarial graft survival analysis at 12 and 24 months

| Variable | *n* | 12 months | (95 % CI) | 24 months | (95 % CI) |
|---|---|---|---|---|---|
| HLA-DRB1: | | | | | |
| 0 mismatches | 47 | 100 | | 100 | |
| 1 mismatch | 191 | 88.7 | (84.2–93.3) | 87.9 | (83.2–92.7) |
| 2 mismatches | 178 | 84.5 | (79.1–89.9) | 81.3 | (75.3–87.4) |
| HLA-A,B: | | | | | |
| 0–2 mismatches | 325 | 89.6 | (86.2–92.9) | 88.7 | (85.1–92.2) |
| 3–4 mismatches | 91 | 83.3 | (75.5–91.0) | 79.1 | (70.4–87.8) |
| Donor age: ≤ 50 years | 356 | 89.7 | (86.6–92.9) | 88.0 | (84.5–91.6) |
| > 50 years | 60 | 79.3 | (68.9–89.8) | 77.3 | (66.3–88.2) |
| Graft number: | | | | | |
| First transplant | 388 | 88.7 | (85.5–91.9) | 86.8 | (83.3–90.3) |
| Retransplant | 28 | 81.2 | (66.2–96.2) | 81.2 | (66.2–96.2) |
| Pre-tx transfusion: 0 | 204 | 90.1 | (86.0–94.2) | 88.7 | (84.1–93.2) |
| > 0 | 212 | 86.4 | (81.7–91.1) | 84.4 | (79.3–89.5) |
| PRA: 0 | 352 | 89.0 | (85.7–92.3) | 86.9 | (83.2–90.6) |
| > 0 | 64 | 83.3 | (73.8–92.8) | 83.3 | (73.8–92.8) |
| Recipient age: ≤ 55 years | 381 | 88.8 | (85.6–92.0) | 86.9 | (83.3–90.4) |
| > 55 years | 35 | 81.9 | (68.7–95.1) | 81.9 | (68.7–95.1) |

**Table 3** Steps in the Cox model stepwise regression analysis

| Step | Variable (level) | Regression coefficient | Standard error | Relative risk | 95 % CI | Chi-square increment | *P* value |
|---|---|---|---|---|---|---|---|
| 0 | | | | | | 21.3 | |
| 1 | HLA-DRB1 (0–2 mismatches) | 0.69 | 0.24 | 2 | 1.2–3.2 | 10 | 0.001 |
| 2 | HLA-A,B (0–2, 3–4 mismatches) | 0.64 | 0.29 | 1.9 | 1.1–3.4 | 4.9 | 0.02 |
| 3 | Donor age (≤ 50 years, > 50 years) | 0.55 | 0.33 | 1.7 | 1.0–3.3 | 4.1 | 0.04 |
| 4 | Graft number (First-tx, re-tx) | 0.36 | 0.53 | 1.4 | 0.5–4.0 | 1.1 | 0.3 |
| 5 | Pre-tx transfusion (0, > 0) | 0.18 | 0.29 | 1.2 | 0.7–2.1 | 0.7 | 0.4 |
| 6 | PRA (0, > 0) | 0.25 | 0.41 | 1.3 | 0.6–2.8 | 0.3 | 0.6 |
| 7 | Recipient age (≤ 55 years, > 55 years) | 0.18 | 0.46 | 1.2 | 0.5–2.9 | 0.2 | 0.6 |

Patient survival was 100 % for zero mismatches, 97.8 % (CI 95.6–99.9) for patients with one mismatch and 93.8 % (CI 89.9–97.6) for patients with two HLA-DRB1 mismatches; 25 % of the deaths among the one mismatch transplants were due to infection and the percentage rose to 45.5 % in the two mismatch transplants.

Table 3 reports the results of the Cox regression analysis. The transplant center variable was not included since a preliminary analysis showed it had no effect on graft survival. Of the variables tested, HLA-DRB1 generic matching was the most significant in influencing graft survival, accounting for 47 % of total $\chi^2$ ($P = 0.001$). HLA-A,B compatibility was the second most influential variable, accounting for a further 23 % of graft survival variability ($P = 0.02$). Finally, donor age was marginally significant, accounting for 19 % of survival variability ($P = 0.04$). Patients with one HLA-DRB1 mismatch had twice as great a risk of returning to dialysis as patients with zero mismatches (RR = 2) and patients with two mismatches had twice as great a risk of graft failure as patients with one mismatch. Similarly, patients with 3–4 HLA-A,B mismatches had a RR of 1.9.

On the basis of the results of the Cox analysis, we investigated the combined effect of HLA-DRB1 and HLA-A,B compatibility on actuarial graft survival (Fig. 1). While transplants with 0–2 mismatches had a 24-month graft survival of 90.7 % (CI 85.1–96.2) and those with 3–4 mismatches had an 88.4 % graft survival (CI 84.3–92.5), those with 5–6 mismatches had a 68.4 % graft survival (CI 55.0–81.8).

Table 4 reports the proportion of patients with no rejection episodes in the 3 months following transplanta-

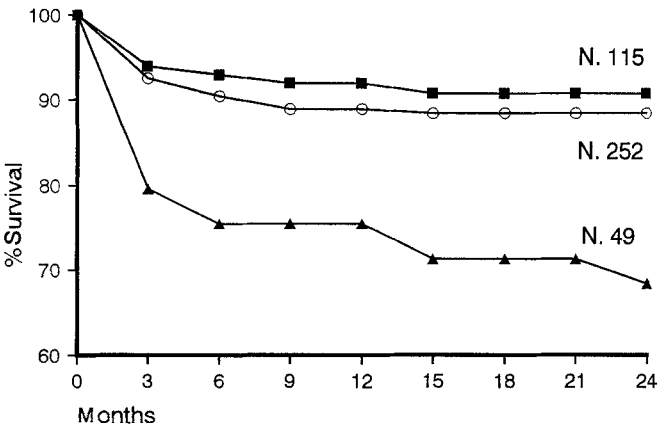


**Fig. 1** Actuarial graft survival according to HLA-A,B, DRB1 mismatches (■ 0–2 mismatches, ○ 3–4 mismatches, ▲ 5–6 mismatches) $P = 0.0001$

tion and the mean creatinine level at the 3rd post-transplant month, according to the number of HLA-DRB1 mismatches for the 198 patients for whom graft function data were available. The probability of experiencing no rejection episodes was different in the three HLA-DRB1 mismatch levels, the most significant difference existing between patients with zero mismatches and those with two mismatches. Rejecting patients with one mismatch received a median of four steroid pulses, whereas those with two mismatches received a median of five steroid pulses in the 3 post-transplant months.

As for the creatinine value at the 3rd month, it increased with HLA-DRB1 mismatches, but a statistical significance was observed only between patients with zero and those with two mismatches.

## Discussion

Molecular biology techniques have had a noteworthy impact on transplantation immunology. There is clear evidence that genomically determined HLA-DR compatibility correlates better with kidney graft survival than serologically determined HLA-DR matching [5, 8, 13, 14, 19]. In a recent study, Opelz et al. analyzed 718 patients with discrepant serology and genomic HLA-DR typing and found that graft survival correlated only with genomic HLA-DR matching [14]. In a study of 91 renal donor-recipient pairs, Hsia and coworkers found a significantly higher graft survival in better HLA-DRB1-matched recipients of cadaver kidney transplants [5]. Kobayashi et al. also reported data suggesting that acute rejection episodes occurred less frequently in patients with zero HLA-DRB1 mismatches than in those with one or two mismatches [8].

In our setting, where a 27% serology/DNA typing discrepancy rate has been recorded [17], an influence

**Table 4** Rejection episodes within the first 3 post-transplant months according to the number of HLA-DRB1 mismatches

| Variables | Number of HLA-DRB1 mismatches | | |
|---|---|---|---|
| | 0 ($n = 25$) | 1 ($n = 87$) | 2 ($n = 86$) |
| Patients with no rejection episodes: | | | |
| Number | 23 | 54 | 35 |
| Proportion (%) | 92 | 62 | 41 |
| 95% confidence intervals | 74–99 | 51–72 | 30–52 |
| Creatinine level at 3rd post-tx month (mg/dl)[a]: | | | |
| Mean | 1.2 | 1.4 | 1.5 |
| 95% confidence intervals | 1.1–1.3 | 1.3–1.5 | 1.4–1.6 |

[a] Reciprocal value was considered for analysis

of HLA-DR compatibility on graft survival became apparent only with DNA typing [19, 20]. In another study in which we investigated the effect of HLA-DRB1 compatibility on patients with functioning grafts for at least 10 years, long-term surviving recipients were significantly better matched than the control group and required less intensive antirejection treatment than less well-matched recipients [18].

The present study strongly indicates that of those factors considered, HLA-DRB1 generic matching is the most important one influencing graft survival, followed by HLA-A,B compatibility and donor age. These results were found both in the univariate as well as in the multivariate analysis. Survival and graft function progressively worsened with increasing HLA-DRB1 mismatches. Fully HLA-DRB1-matched patients had a better transplant outcome, as demonstrated by the 100% 24-month graft survival, the lower rejection frequency rate, and the reduced creatinine level compared with mismatched transplants. HLA class I matching did have an effect on graft survival, as shown by the Cox regression analysis, and it seems independent from HLA-DRB1 compatibility; in fact, Table 1 shows that HLA-A,B mismatches were similarly distributed in the three groups of patients divided according to HLA-DRB1 mismatches. Furthermore, including HLA-A,B in the Cox model significantly improved the fit ($P = 0.02$).

The present study indicates that HLA-DRB1 generic typing is sufficient to improve the correlation between matching and transplant outcome, a finding that is in agreement with that of Opelz and coworkers [14]. Nevertheless, the importance of high resolution HLA-DRB1 matching must be investigated.

Donor age was marginally significant, both in the univariate and in the multivariate analysis, suggesting that the use of kidneys from donors above 50 years of age implies an additional risk.

We are aware that our data warrant further comments. Firstly, it was impossible to investigate an HLA effect in subsets such as regrafts and sensitized subjects

due to the small number of patients. Secondly, graft function data were available for only approximately 50 % of the patients. Moreover, since HLA-DRB1 was not used as a kidney allocation criterion, there were few better-matched recipients, not allowing definite conclusions to be drawn.

These results prompted us to design a prospective pilot study that foresees typing of the patients on the waiting list with SSO typing; this technique was chosen because of reliability and the ease of result interpretation for HLA-DRB1 generic typing. For prospective cadaver donor typing, the PCR-sequence-specific primers (SSP) technique [12] was adopted because it was found to be fast and accurate.

In conclusion, the data presented here suggest that in the NITp, as in other transplant programs, prospective HLA-DRB1 typing will probably improve the outcome of cadaver kidney transplants, allowing better use of this rare resource.

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