
# Shorter Cold Ischemia Time in Deceased Donor Kidney Transplantation Reduces the Incidence of Delayed Graft Function Especially Among Highly Sensitized Patients and Kidneys From Older Donors

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## ABSTRACT

**Background.** Long cold ischemia time (CIT) is the most important factor contributing to delayed graft function (DGF) after kidney transplant. Improvements in pretransplant procedures may reduce CIT and improve clinical outcome.

**Materials and Methods.** Pretransplant histocompatibility tests were modernized at our laboratory in 2015, leading to significant decrease of time consumed for these enabling earlier surgery. The effects of this on kidney transplant CIT, DGF, and other clinical outcomes were studied. The study population consisted of 896 consecutive deceased donor kidney recipients, of which 442 patients received a transplant with the old crossmatch and 454 received a transplant with the new crossmatch.

**Results.** CIT shortened from mean 20 hours 6 minutes to 15 hours 52 minutes ($P < .001$). The incidence of DGF was significantly reduced from 31% to 24% ($P = .02$). Reduction in the frequency of DGF was more pronounced among the highly sensitized patients (53% to 28%, $P = .01$) or in patients with pretransplant donor-specific antibodies (50% to 20%, $P = .002$) and among patients who received kidneys from donors older than 65 years (38% to 27%, $P = .04$).

**Conclusions.** Process optimization that reduces CIT decreases occurrence of DGF, especially in highly sensitized patients and patients who receive kidneys from older donors.

INCREASED cold ischemia time (CIT) is the most important factor contributing to delayed graft function (DGF) after kidney transplant [1], and continuous efforts are aimed to reduce unnecessary delays in the process of kidney transplant from a deceased donor. Prospective complement-dependent cytotoxicity crossmatching is time consuming and is one of the main causes for the prolonged transplant CIT. Although virtual crossmatching may partly

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replace the need for prospective cytotoxic crossmatching, it still remains the gold standard in successful kidney transplant, especially in sensitized patients [2–4].

Originally spleen or lymph nodes of deceased donors were used to yield sufficient number of both B and T cells to perform a reliable cytotoxic crossmatch, but with the more specific techniques to purify T and B cell populations, peripheral blood is now widely used as a source. This enables prospective crossmatching to be started even before the deceased donor organ retrieval operation and thus may reduce CIT significantly, as has been shown by others [5].

Our institution serves as the only transplant center for a nationwide relatively large geographic area, with long distances for patients to travel to the transplant operation. With an unacceptably high frequency of DGF after deceased donor kidney transplant reaching almost 40% in our institution [6], an initiative was launched to optimize CIT to reduce the occurrence of DGF. The aim of this study was to assess the possibility of improving clinical outcomes with enhancing the cooperation between the transplant center and histocompatibility laboratory and to find possible subgroups that would benefit the most from reductions in cold ischemia time.

## MATERIALS AND METHODS

### Donors and Patients

All donors and patients were categorized into group 1 (transplant performed before October 12, 2015, a time of introduction of new crossmatching method) or group 2 (after October 12, 2015). Our institution is the only transplantation center in Finland and is also responsible for all of the organ procurement operations. According to Scandiatransplant exchange rules (http://www.scandiatransplant.org/organ-allocation/guidelines), some of the deceased donor organs are transplanted in other countries within the Scandiatransplant area. All donors were brain dead because donors after circulatory death are currently not used in Finland. Machine perfusion was not used in any of the transplants.

All deceased donor kidney transplant recipients in our center who received transplants during a 2-year period before and after the histocompatibility testing changes, that is, patients between October 9, 2013, and October 16, 2017 (N = 896), were included in this prospective follow-up study of an inception cohort. All patients were either on hemodialysis or peritoneal dialysis before transplant, and no preemptive deceased donor kidney transplants were performed during the study period. Altogether 442 patients received a transplant in the selected era of the old crossmatch (group 1), and 454 received a transplant with the new crossmatch (group 2). Eighty-three transplanted kidneys were from donors of other Scandinavian countries: 43 and 40 in groups 1 and 2, respectively. Data about patient characteristics, CIT, the occurrence of DGF, and possible rejection episodes were gathered from the National Transplant Registry, to which follow-up data from transplant patients are prospectively collected, as obliged by law. DGF was defined as the need for post-transplant dialysis during the first week after transplant. Data about pretransplant donor-specific HLA antibodies (DSAs) were collected from Finnish Red Cross Blood Service's laboratory records.

### Donor-Recipient Matching Protocol, Cytotoxic Crossmatching, and HLA Typing

Recipients for deceased donor kidney transplant were selected based on a predesigned protocol taking into account ABO compatibility and HLA-A, -B, and -DRB1 match, with emphasis on DRB1 matching, waiting time, clinical evaluation, and negative cytotoxic crossmatch.

On October 12, 2015, the method using density gradient-purified spleen cells for crossmatching was replaced with purification of peripheral blood T and B cells for crossmatching using RosetteSep kits (Stemcell Technologies, Vancouver, Canada), performed according to manufacturers' instructions. Internal validation of the use of RosetteSep-separated peripheral T and B cells for crossmatch showed no relevant differences compared with splenocyte crossmatch in the specificity and sensitivity (89% and 82%, respectively). When the crossmatching methods were compared with the cumulative (the sum of all individual DSAs above 1000 mean fluorescence intensity [MFI]) DSAs of more than 3000 MFI as standard, specificity and sensitivity were similar (81% and 67% in peripheral T and B cell method, and 81% and 71% in splenocyte method, respectively). Splenocyte crossmatch was performed using the 2 latest patient sera (1 μL, 5 μL, and 1 μL 1/10 dilution with 2 μL standard rabbit complement and 1 μL with 4 μL complement). The incubation times of the serum and cells were 20 minutes, after complement 60 minutes, and with color 20 minutes. Dithiothreitol treatment was used if IgM antibodies were previously detected. All cases having more visually detected dead cells compared with negative control were called positive. Peripheral T and B cell crossmatches were performed similarly, but in B cells the cutoff for positive level was increased to more than 20% of dead cells. Also, complement incubation period was reduced to 30 minutes.

Spleen retrieval was performed simultaneously with transplantable organs, but peripheral blood samples were usually delivered to the laboratory before donor operation. Patient sera used for initial crossmatches were collected previously for HLA antibody analyses and stored at the laboratory.

HLA-A, -B, and -DRB1 loci of the patients were determined with Luminex using One Lambda LABType kits (One Lambda Inc, Canoga Park, Calif, Untied States). Before June 2015, the donor HLA typing for HLA-A, -B, -C, -DRB1, and -DQB1 was performed with sequence-specific primers (One Lambda Inc) and with complement-mediated lymphocytotoxicity test (Biotest, Rockaway, NJ, United States).

After introduction of LINKSEQ qualitative polymerase chain reaction method with HLA-ABCDRDQA1DQB1DP 384 Typing Kit (Linkage Biosciences, San Francisco, Calif, United States) in June 2015, HLA-DPA1, -DPB1, -DQA1, and -DRB3-5 have also been determined at low resolution level. High-resolution results have been recorded whenever possible either because of adequate typing resolution or based on well-known haplotypes. HLA typing was performed according to donor center rules in 83 cases with exchanged kidney, and at least HLA-A, -B, -C, -DRB1, and -DQB1 loci were determined at low-resolution level in all donors.

Time spent for HLA typing including splenocyte (group 1) or T and B cell (group 2) crossmatches for possible thoracic organ or pancreas transplant recipients, time spent during waiting for the kidney transplant candidate list for the crossmatches from the transplantation office, and time spent for kidney transplant crossmatches with splenocytes in group 1 and with T and B cells in group 2 were gathered from all local donor cases with adequate laboratory records in 2014 (group 1, N = 101) and 2016 (group 2, N = 104). This sample approach was selected to ensure that all personnel

**Table 1. Time Spent (min) for Different Steps During Histocompatibility Tests in 2 Selected Time Periods: 2014 (Group 1) and 2016 (Group 2)**

| | Group 1 (N = 101) | Group 2 (N = 104) | *P* Value |
|---|---|---|---|
| Start HLA typed* | | | |
| Mean (SD) | 277 (58) | 229 (62) | < .001‡ |
| Median (range) | 265 (165-450) | 210 (120-410) | |
| List waiting | | | |
| Mean (SD) | 100 (74) | 107 (83) | .6 |
| Median (range) | 85 (15-435) | 88 (5-435) | |
| Crossmatches† | | | |
| Mean (SD) | 531 (200) | 204 (59) | < .001‡ |
| Median (range) | 490 (235-1155) | 190 (125-590) | |
| Laboratory work time | | | |
| Mean (SD) | 807 (213) | 430 (87) | < .001‡ |
| Median (range) | 765 (500-1500) | 410 (305-780) | |
| Total time | | | |
| Mean (SD) | 907 (221) | 536 (120) | < .001‡ |
| Median (range) | 870 (520-1615) | 515 (345-1015) | |

*Includes locally performed HLA typing and crossmatches for thoracic organ and pancreas transplant recipients.
†Includes crossmatches for kidney transplant recipients.
‡$P < .05$.

working on-call duties were familiar with the old and new methods. Mean total time consumed for histocompatibility testing decreased from 15 hours 7 minutes to 8 hours 56 minutes ($P < .001$). Effects of methodologic changes to time spend for histocompatibility test are shown in detail in Table 1.

## DSA Analyses

One Lambda Labscreen mixed- and single-antigen beads with Luminex were used for HLA antibody screening and identification with the use of HLA Fusion software (One Lambda Inc). A normalized MFI cutoff point of 1000 was used for positivity in single antigen analyses. Donor specificity of all positive antibodies were evaluated, and MFI values of detected DSAs were recorded, with the exception of HLA-DP antibodies of those cases where donor HLA-DP type was unknown (n = 12 and n = 4 in groups 1 and 2, respectively).

## Immunosuppression

Immunosuppression was a combination of cyclosporine or tacrolimus, mycophenolate, and steroids. Induction was not used in the majority of the patients, but induction with basiliximab or antithymocyte globulin (ATG) was given to patients with higher immunologic risk, such as patients with poor HLA match, increased level of panel-reactive antibodies (PRAs), or known pretransplant DSAs with clinician's discretion. Virtual crossmatch results were usually not available at the time of transplant, and in addition to induction therapy, no desensitization was used perioperatively for patients with DSAs. Trough level targets for cyclosporine and tacrolimus were similar for all patients regardless of the induction used, and no differences were made to the policies during the study period. Immunosuppression protocol has been previously reported in detail [7].

## Statistics

Continuous variables were compared using the Mann-Whitney *U* test. Categorical variables were compared using the $\chi^2$ test. Survival was analyzed with the Kaplan-Meier method, and differences between the groups were analyzed with the log-rank test. The independent impact of CIT on the risk of DGF was analyzed with multivariable binary logistic regression analysis, with the occurrence of DGF as the binary outcome. All covariates with $P < .1$ in univariable analysis were selected to the final multivariable model. SPSS version 21 (IBM, Armonk, NY, United States) was used for analysis. *P* values < .05 were considered statistically significant.

## Ethical Aspects

This study was based purely on registry data and was approved by the scientific committee of the Finnish Red Cross Blood Service and by the Hospital District for Helsinki and Uusimaa (HUS/269/2017). The coordinating ethical committee for the Hospital District of Helsinki and Uusimaa has also approved the use of patient record data for scientific studies (42/13/03/00/11). The persons involved in this study were treated in a manner in accordance with both the Declaration of Helsinki and the Declaration of Istanbul.

**Table 2. Donor and Patient Characteristics**

| | Group 1 (N = 442) | Group 2 (N = 454) | *P* Value |
|---|---|---|---|
| Donor age, mean (SD), y | 56 (16) | 55 (16) | .92 |
| Patient age at time of transplant, mean (SD), y | 52 (15) | 53 (15) | .13 |
| Patient sex, % female | 37 | 33 | .19 |
| Retransplant, % | 16 | 14 | .53 |
| Class I HLA mismatch, mean, No. | 2 | 2 | .84 |
| Class II HLA mismatch, mean, No. | 1 | 1 | .92 |
| Pretransplant peritoneal dialysis, No. (%) | 161 (36) | 138 (30) | .30 |
| Tacrolimus based immunosuppression, No. (%) | 177 (40) | 226 (50) | .03* |
| Induction immunosuppression, No. (%) | 73 (17) | 121 (27) | < .001* |
| ATG | 33 (7) | 61 (13) | |
| Basiliximab | 40 (9) | 60 (13) | |
| Sensitized pretransplant (%) | 42 | 35 | .02* |
| Highly sensitized (PRA > 80%) pretransplant (%) | 12 | 13 | .36 |
| Pretransplant DSA (%) | 10 | 12 | .18 |

Abbreviations: ATG, antithymocyte globulin; DSA, donor-specific HLA antibody; PRA, panel-reactive antibody.
*$P < .05$.

**Table 3. Clinical End Points Among Patients Who Received Transplants During the Old Crossmatch (Group 1) or the New Crossmatch (Group 2)**

| | Group 1 (N = 442) | Group 2 (N = 454) | *P* Value |
|---|---|---|---|
| Cold ischemia time, mean (SD), h | 20 (4) | 16 (6) | < .001* |
| Nonfunction, No. (%) | 8 (1.8) | 8 (1.8) | > .99 |
| DGF, No. (%) | 139 (31) | 111 (24) | .02* |
| Dialysis sessions needed post Tx in patients with DGF, mean (SD), No. | 5 (4) | 4 (4) | .23 |
| Acute cellular rejection, No. (%) | 48 (11) | 57 (13) | .47 |
| Acute humoral rejection, No. (%) | 22 (5) | 15 (3) | .24 |
| Plasma creatinine at the end of follow-up, mean (SD), μmol/L | 147 (125) | 150 (91) | .72 |
| 1-year graft survival, No. (%) | 414 (94) | 432 (95) | .39 |
| 1-year patient survival, No. (%) | 421 (95) | 440 (97) | .23 |

Abbreviations: post Tx, post transplant; DGF, delayed graft function.
*$P < .05$.

## RESULTS

### Patients

Patients included in the study are described in Table 2. Patients in group 1 were more frequently sensitized before transplant ($P = .02$), but the frequency of highly sensitized patients (PRA > 80%) or patients with pretransplant DSAs did not differ between the groups. Patients in group 2 received more frequently tacrolimus-based immunosuppression and induction therapy with ATG or basiliximab, but no other significant differences were found in the baseline characteristics between the patients in groups 1 or 2. At the end of follow-up on December 31, 2017, a total of 845 grafts (94%) were functioning, and 866 patients (96%) were alive.

### Clinical End Points

Table 3 compares the clinical end points between patients in groups 1 and 2. CIT reduced from mean 20 hours 8 minutes (SD, 4 hours 36 minutes) in group 1 to 15 hours 50 minutes (SD, 5 hours 12 minutes) in group 2 ($P < .001$). Figure 1 shows the distribution of CIT in the 2 groups. Among patients in group 1, only 17% of patients received transplants within 16 hours of CIT compared with 53% in group 2.

Altogether 16 patients (8 patients in each group) experienced early graft loss during the first week after transplant owing to vascular thrombosis without graft function or primary nonfunction of unknown cause. No cases of hyperacute rejection were observed. The frequency of DGF was 139 of 442 (31%) in group 1 compared with 111 of 454 (24%) in group 2 ($P = .02$). No significant differences were seen in the number of dialysis sessions needed among patients with DGF. No differences were seen in the frequency of biopsy-proven acute cellular or humoral rejections or in graft function at the end of follow-up. Similarly, no differences were seen in graft or patient survival between the groups.

### Sensitivity Analyses

*Sensitized patients.* When only patients with known DSAs at the time of transplant were included in the analyses, the reduction in the frequency of DGF was even more pronounced: 22 of 44 (50%) in group 1 compared with 11 of 55 (20%) ($P = .002$). A similar effect was seen when only highly sensitized patients (pretransplant PRA > 80%) were included because the frequency of DGF was reduced from 28 of 52 (53%) in group 1 to 17 of 59 (28%) ($P = .01$). The reduction in CIT was similar in patients with DSAs at the time of transplant or highly sensitized patients, from mean 20 hours in group 1 to mean 14 hours or 15 hours in group 2, respectively. To avoid possible bias in the severity of

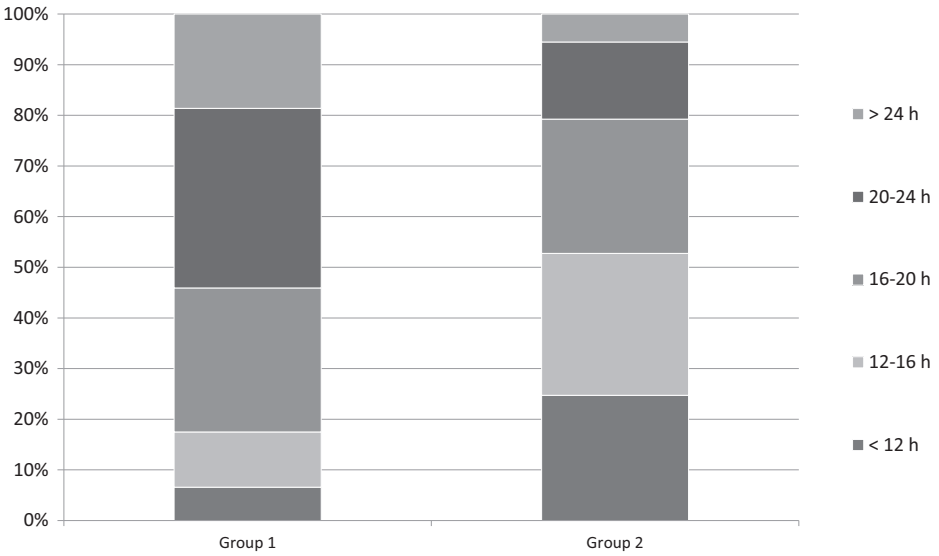


**Fig 1.** Distribution of cold ischemia times before (group 1) and after (group 2) the change of the laboratory procedures.

**Table 4. Characteristics of the Patients With Donor-Specific Antibodies**

| Patients with DSAs | Group 1 (N = 44 of 442, 10%) | Group 2 (N = 56 of 454, 12%) | *P* Value |
|---|---|---|---|
| No. of DSAs, No. (%) | | | .66 |
| 1 | 24 (55) | 27 (48) | |
| 2 | 8 (18) | 15 (27) | |
| 3 | 7 (16) | 9 (16) | |
| ≥ 4 | 5 (11) | 5 (9) | |
| DSAs against, No. (%) | | | .42 |
| Class I | 28 (64) | 30 (54) | |
| Class II | 9 (20) | 12 (21) | |
| Both | 7 (16) | 14 (25) | |
| Cumulative MFI, median (range) | 4986 (1061-31190) | 8208 (1001-54363) | .24 |
| Patients on tacrolimus (vs cyclosporine), No. (%) | 33 (75) | 43 (78) | .81 |
| Patients receiving induction, No. (%) | | | .06 |
| Basiliximab | 12 (27) | 15 (27) | |
| ATG | 4 (9) | 15 (27) | |
| Cold ischemia time, mean (SD), h | 20 (4) | 14 (4) | < .001 |
| Patients with acute rejection, No. (%) | 9 (20) | 17 (30) | .26 |

Abbreviations: ATG, antithymocyte globulin; DSA, donor-specific antibody; MFI, mean fluorescence intensity.

sensitization between the groups, both the highly sensitized group (PRA > 80%) and patients with pretransplant DSAs are compared in Tables 4 and 5. No significant differences were recorded between the groups regarding number of DSAs, the distribution of DSAs between HLA class I and II antibodies, median cumulative MFI, the frequency of acute rejection, or the immunosuppression used. A statistically nonsignificant trend was seen toward more use of ATG in patients with pretransplant DSAs in the group 2, whereas the cumulative median MFI of the DSAs was slightly higher in group 2.

*Impact of donor age.* When only patients who received a kidney from a donor 65 years or older were included, the frequency of DGF was 50 of 130 (38%) in group 1 and 43 of 160 (27%) in group 2 ($P = .04$). The reduction in CIT was similar in patients who received kidneys from donors 65 years or older compared with younger donors, from mean 21 hours in group 1 to mean 17 hours in group 2, respectively ($P < .001$). On the contrary, when patients received a kidney from a donor younger than 45 years, no significant difference was seen in the frequency of DGF: 16 of 78 (21%) in group 1 and 17 of 110 (15%) in group 2 ($P = .4$). Among patients who received a graft from a donor younger than 45 years with a CIT less than 12 hours, only 3 of 54 (6%) developed DGF. On the other hand, among the 290 patients who received a kidney from a donor 65 years or older, only 21 patients received transplants with a CIT of less than 12 hours, and only 1 of them (5%) developed DGF.

### Multivariable Model of Risk Factors for DGF

A binary logistic regression model was built to analyze the independent effect of cold ischemia on DGF. Covariates with $P < .1$ in univariable analysis were selected to the final multivariable model shown in Table 6. Odds ratio for CIT (per 1 hour increase) in univariable model was 1.12 (95% CI, 1.09-1.16, $P < .001$). When CIT was categorized to 5 categories (< 12h, 12-15.9 h, 16-19.9 h, 20-23.9 h, and > 24 h), each incremental increase in CIT was associated with an increased risk for DGF (odds ratio, 1.62; 95% CI, 1.42-1.84; $P < .001$). Increased CIT remained an independently significant risk factor in the adjusted model, with an odds ratio of 1.11 for each 1-hour increase in CIT. No significant first-degree interactions were seen between CIT and the other variables. In addition to the variables included in the multivariable model shown in Table 6, variables such as the use of tacrolimus (vs cyclosporine), HLA mismatch, or recipient or donor sex were tested but were not associated with the risk of DGF in univariable analyses.

## DISCUSSION

In this study, we were able to show that CIT can be reduced by optimizing histocompatibility testing, leading to reduced incidence of DGF. In addition, because other treatment policies remained unchanged during the study period, we had a possibility to examine the independent impact of CIT on the risk of DGF.

In addition to CIT, which is regarded as the most important risk factor for DGF [1], other factors contribute to the risk of DGF after deceased donor kidney transplant, such as donor age [8], presence of DSA [9], pretransplant hemodialysis [6], management of the brain-dead donor [10,11], and possible use of machine perfusion during the cold storage [12]. The extent of ischemia-reperfusion injury to the graft causing DGF is likely a multifactorial process in which the weight of the individual components is not entirely discovered because many of the factors are interrelated. For example, highly sensitized patients may have their kidney shipped from other centers and may need confirmatory prospective crossmatching, resulting in increased CIT. Our study provides important insights to the actual contribution of CIT to the risk of DGF. In our analysis, donor age was an important risk factor for DGF, and among patients with a young donor and short CIT (< 12 h), the frequency of DGF was very low, only 6%. On the other hand, we were able to show that reducing CIT might be even more important among kidneys transplanted from older donors because the reduction in the occurrence of DGF was more pronounced among donors 65 years or older. Other studies have reported similar findings of the increased susceptibility to cold storage of kidneys from older donors [13–15].

Another subgroup of patients who seemed to achieve more benefit from reduction of CIT were highly sensitized patients because we found that the reduction in the frequency of DGF was substantial among highly sensitized

**Table 5. Characteristics of the Highly Sensitized Patients**

| Highly Sensitized Patients (PRA > 80%) | Group 1 (N = 53 of 442, 12%) | Group 2 (N = 59 of 454, 13%) | *P* Value |
|---|---|---|---|
| Patients with DSA, No. | 26 | 33 | .57 |
| No. of DSAs, No. (%) | | | .97 |
| 1 | 8 (15) | 10 (17) | |
| 2 | 7 (13) | 10 (17) | |
| 3 | 6 (11) | 8 (14) | |
| ≥ 4 | 5 (9) | 5 (8) | |
| DSAs against, No. (%) | | | .64 |
| Class I | 17 (32) | 19 (32) | |
| Class II | 5 (9) | 7 (12) | |
| Both | 3 (6) | 7 (12) | |
| Cumulative MFI, median (range) | 8825 (1114-31190) | 12992 (1001-53362) | .11 |
| Patients on tacrolimus (vs cyclosporine), No. (%) | 43 (81) | 49 (83) | .81 |
| Patients receiving induction, No. (%) | | | .29 |
| Basiliximab | 13 (25) | 20 (34) | |
| ATG | 7 (13) | 11 (19) | |
| Cold ischemia time, mean (SD), h | 20 (4) | 15 (4) | < .001 |
| Patients with acute rejection | 11 (21) | 14 (24) | .82 |

Abbreviations: ATG, antithymocyte globulin; DSA, donor-specific antibody; MFI, mean fluorescence intensity; PRA, panel-reactive antibody.

patients or patients with DSAs at the time of transplant, from 52% to 28% and 50% to 20%, respectively. We have previously demonstrated that the presence of pretransplant DSAs increases the risk of DGF after kidney transplant [9]. The increased immunogenicity in the graft caused by ischemia-reperfusion injury [16] might be even more detrimental among patients with already baseline higher risk for immunologic events. In our current analysis, pretransplant DSA was not an independent risk factor for DGF, which might be explained by different definitions of DGF in our previous study and the fact that the use of induction immunosuppression has been increasing in our institution since the previous study. In fact, patients in group 2 were more likely to receive induction immunosuppression, which might be a confounding factor when assessing the risk of DGF. Selection of the induction treatment was based on clinician's discretion to choose between basiliximab and ATG. Induction immunosuppression, however, was not independently associated with the risk of DGF in the current study.

In their excellent report, Aubert et al have shown that the presence of DSAs and long CIT are both detrimental to extended criteria donor kidney transplant survival [17]. Our results of the beneficial effect of CIT reduction especially in patients with DSAs or kidney transplant from an older donor are in line with those findings. However, our end point was DGF, not graft of patient survival, and our results do not necessarily mean that our patients with a higher frequency of DGF may also have poorer outcome in the long run or that reduced CIT in these subgroups actually may enhance graft of patient survival. In fact, no difference in short-term graft or patient survival, graft function in the long term, or the frequency of acute rejection could be detected between the groups in our current study, suggesting that the reduced frequency of DGF may not translate to long-term benefit of the transplant. Conflicting data exist in the literature about the association of DGF with long-term outcomes, and not all data support the harmful long-term effects of DGF [15,18].

Similar findings of logistical factors influencing CIT have been reported by others [3–5,19]. In a prospective multicenter study from the United Kingdom, Shrestra et al showed that the use of donor peripheral blood for crossmatching was associated with reduced CIT. Frequencies of DGF, however, were not included in this report. The shortest CITs were reported in patients with transplants

**Table 6. Multivariable Model of the Impact of the Cold Ischemia Time for Delayed Graft Function After Kidney Transplant**

| | Univariable Model | | | Multivariable Model | | |
|---|---|---|---|---|---|---|
| | OR | 95% CI | *P* Value | OR | 95% CI | *P* Value |
| Cold ischemia time, h | 1.12 | 1.08-1.16 | < .001 | 1.11 | 1.07-1.15 | < .001 |
| Donor age, y | 1.02 | 1.01-1.03 | < .001 | 1.02 | 1.01-1.03 | .004 |
| Retransplant | 1.19 | 1.31-2.81 | .001 | 1.52 | 0.94-2.46 | .09 |
| Highly sensitized status | 1.90 | 1.26-2.86 | .002 | 1.88 | 1.14-3.10 | .01 |
| Recipient female sex | 0.72 | 0.53-0.97 | .03 | 0.69 | 0.51-0.95 | .02 |
| Induction therapy | 0.56 | 0.38-0.83 | .004 | 0.69 | 0.43-1.10 | .12 |
| Pretransplant peritoneal dialysis | 0.33 | 0.21-0.50 | < .001 | 0.38 | 0.24-0.60 | < .001 |

Abbreviation: OR, odds ratio.

performed with virtual crossmatching and no prospective cytotoxic crossmatching [5]. A major limitation to this policy is that virtual crossmatching is usually only suitable for nonsensitized patients or patients with only limited sensitization and low number of unacceptable HLA antigens. One-third of our population in the current study were sensitized pretransplant and not optimally suitable for virtual crossmatching, and these patients are at the highest risk of increasing CIT and DGF.

Our decision to use potentially less sensitive peripheral blood leukocytes for cytotoxic crossmatch could lead to increased rate of false negative results when the presence of DSAs is used as the definition of true positive. It is known that especially low MFI level Luminex-based HLA-antibody findings are clinically too sensitive [20–22]. However, occasionally high-level DSAs are also seen in crossmatch negative patients. We have accepted this since these patients usually have prolonged waiting time, are highly sensitized, and have a very limited chance of finding virtual crossmatch negative transplants. Flow cytometric crossmatch could improve the accuracy of crossmatching. However, previous studies from our laboratory and our experience has shown that flow cytometric crossmatch correlates rather well with virtual crossmatch [23]. Therefore, adding flow cytometric crossmatch to our protocol might only add another layer of studies but might not be cost effective or clinically helpful. Naturally, we aim to add virtual crossmatch to our protocol as an additional decision-making tool for clinicians. Despite less sensitive biological crossmatch methods, no hyperacute rejection episodes of kidney transplants have been detected at our clinic after negative cytotoxic crossmatch, neither in the previous era with splenocyte crossmatching nor in the current era of peripheral blood leukocyte crossmatching. The concept of using peripheral blood cells for crossmatching is not new and has been successfully adopted by several centers. Thus, our findings regarding the crossmatching policy are not novel, and reduction in CIT and the frequency of DGF could be expected. However, the purpose of this study was more to demonstrate that changes in policies and cooperation between the transplant center and histocompatibility laboratory can lead to meaningful improvement in clinical outcomes.

Our study has some limitations of note. Because this was a single-center study, the findings might not be applicable to other kidney transplant populations. In addition, although treatment policies in our center have remained stable since October 2013, the use of tacrolimus and induction therapy have increased in the latter patient group, and we cannot rule out unidentified confounding factors. Only limited data were available regarding the deceased donors, and some variables associated with the risk of DGF (such as donor body mass index, cause of death, or data on management of the deceased donor) could not be adjusted for, limiting our analyses. However, no changes occurred in donor acceptance or management policies during the study period, and likely no differences exist between the study groups regarding the management or selection of the brain-dead donors. On the other hand, our study includes a relatively large number of kidney transplants from deceased donors within a short time-period, allowing us to analyze the independent impact of CIT on the risk of DGF.

## CONCLUSIONS

Enhanced histocompatibility testing methods can help to reduce CIT and the occurrence of DGF in kidney transplantation, showing that major improvements in patient care and results of kidney transplant can be made by optimizing the crosstalk between the histocompatibility testing laboratory and the transplantation center. Highly sensitized patients and patients who receive kidney from older donors seem to benefit more from the reduction in CIT.

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