Influence of CDC-XM and HLA compatibility on clinical outcomes in kidney transplant recipients during the post-operative recovery period: A retrospective analysis

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Abstract. Kidney transplantation remains the preferred treatment for patients with end-stage kidney failure. Complement-dependent cytotoxicity (CDC) crossmatch (CDC-XM) and human leukocyte antigen (HLA) typing are two important methods of donor-recipient matching prior to kidney transplantation. The purpose of the present study was to explore the effects of CDC-XM levels and HLA matching on early post-operative clinical outcomes in kidney transplant recipients. A total of 112 consecutive recipients who underwent allogeneic kidney transplantation were selected and their data collected, including pre-operative general information, indicators associated with renal function, red blood cell and white blood cell counts, the blood glucose level at each follow-up time point and both the incidence of adverse events following transplantation and their risk factors. During the follow-up period, statistical methods were used to compare and systematically analyze the differences in clinical indicators and adverse events between each groups. In each different groups that were assigned for the CDC-XM levels and HLA matching, the differences in the clinical indicators between the groups during the follow-up period were mainly centered on the first week post-transplantation, with the greatest differences being identified for the renal function-associated indicators, whereas the observed recovery was essentially comparable between 1-6 months. According to the multivariate analysis, recipients of age ≥40 years and with a BMI ≥25 tended to have an increased risk of delayed graft function (DGF), whereas the risk was reduced when the organ had been donated by a living donor and also when the number of HLA mismatches was 0-2. In conclusion, the present study showed that CDC-negativity and improved HLA matching help to promote the recovery of renal function in kidney transplant recipients during the early post-operative period. Patients who met the conditions of CDC-negativity and fewer HLA mismatches had faster and improved early postoperative recovery of renal function and a lower incidence of DGF. Furthermore, in a multifactorial analysis of DGF, recipient age, recipient BMI, donor type and HLA mismatch were found to be important risk factors for DGF.

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Introduction

End-stage renal disease (ESRD) remains a global health concern. Given the application of new immunosuppressive agents and the continuous improvement of organ transplantation technology, allogeneic kidney transplantation has become the best alternative therapy for the treatment of ESRD (1), which may lead to improvements in the survival rate and quality of life of patients compared with continuous dialysis (2). However, potential kidney transplant recipients often develop anti-human leukocyte antigen (HLA) antibodies due to previous transplants, blood transfusions and pregnancy. This HLA pre-sensitization status not only decreases the success rate of transplant matching, but it also markedly increases the risk of acute rejection (AR) following transplantation. Antibody-mediated rejection is the main cause of chronic kidney graft injury, which is characterized by the existence of donor-specific antibodies (DSA) in kidney transplant recipients and which greatly limits the functional recovery and long-term survival of kidney grafts (3,4). Therefore, it is essential to test the sensitivity of kidney transplant

patients prior to transplantation. The pre-transplant immune risk assessment includes evaluations of pre-existing HLA antibodies and non-HLA antibodies in the recipient, HLA compatibility, complement-dependent cytotoxicity (CDC) crossmatch (CDC-XM), immune memory cells, among other factors. CDC-XM and HLA typing are critical for evaluating donor-recipient compatibility, for minimizing graft rejection and for improving transplant outcomes in solid organ transplantation.

CDC-XM is an important laboratory test that is administered prior to organ transplantation to determine the likelihood of AR through detecting the presence of antibodies in the recipient's body that are able to bind to HLA on the surface of lymphocytes, thereby activating the complement system. In CDC-XM, CDC-positivity is a contraindication for kidney transplantation. The HLA system has a crucial role in regulating the body's immune response, which is mediated through presenting antigens and distinguishing between 'self' and 'non-self', so as to effectively resist the invasion of pathogens. It involves a set of cell-surface antigen-presenting proteins categorized as class I and II major histocompatibility complex molecules (MHC I/II). Humans possess three class I (A, B and C) antigens that are present on all nucleated cells and three class II (DP, DQ and DR) antigens that are present only on antigen-presenting cells and endothelial cells. HLA-A, -B and -DRB1 heterodimers are responsible for the majority of the polymorphisms in HLA, making these heterodimers the primary focus of HLA matching in the allocation of donor kidneys for transplantation (5).

In kidney transplantation, lower levels of CDC-XM and improved HLA matching have been shown to be associated with improved graft survival, along with the post-operative administration of lower dosages of immunosuppressive agents, a reduced incidence of immunosuppressive side effects and a lower degree of sensitization. Currently, recipients with CDC >10% tend to be excluded from studies in Asia and relatively few studies have been published on the short-term effects of CDC and HLA matching in kidney transplantation. Therefore, the aim of the present study was to analyze the effect of CDC-XM levels and HLA matching on early clinical outcomes in kidney transplant recipients.

Patients and methods

Patients and study cohort. This retrospective study included 112 recipients who underwent kidney transplantation between June 2022 and June 2023 at The First Affiliated Hospital of Anhui Medical University (Anhui, China). Ethical approval for The present study was granted by The Ethics Committee of The First Affiliated Hospital of Anhui Medical University (Anhui, China; approval no. PJ2024-05-30). The inclusion criteria for the patients were as follows: i) The patient had received kidney transplantation for the first time; ii) the patient was aged 18-60 years; and iii) the patient was administered a triple immunosuppressive regimen of tacrolimus (TAC) + mycophenolate mofetil (MMF) + glucocorticoids. The exclusion criteria were: i) The patient had already received multiple kidney transplants or combined multi-organ transplants; ii) the patient was <18 or >60 years of age; and iii) patients were with combined infectious diseases, such as viral hepatitis, tuberculosis or acquired immunodeficiency syndrome. All enrolled kidney transplant recipients were screened pre-operatively for low-resolution HLA typing, anti-HLA antibodies and anti-MHC class I-associated molecule A (MICA) antibodies and CDC-XM. The present study was divided into two parts, the first being the prognostic effect on kidney transplant patients based on CDC-XM levels and/or HLA matching and the second part was to identify risk factors for DGF after kidney transplantation. The results were statistically analyzed independently and details of the patients selected from the database are shown in Fig. 1.

CDC-XM analysis. Lymphocytes isolated from the peripheral blood of live kidney donors or the spleen of cadaveric kidney donors were co-incubated with recipient serum on Terasaki plates (One Lambda, Inc.) using Ficoll-Hypaque density gradient centrifugation (400 x g, room temperature, 22 min) and positive and negative controls were set up on Terasaki plates for quality control. Rabbit complement was added to each well for co-incubation for 60 min at 37˚C, subsequently followed by the addition of 5 μl fluorescence terminator FluoroQuench™ (One Lambda, Inc.), the plates were placed under an inverted phase-contrast fluorescence microscope to count the number of dead lymphocytes from a total of 300 lymphocytes in order to assess cell death in the presence of complement and then the results were expressed as percentages (%). The CDC-XM data collected in the present study were based on the results of the final pre-operative examinations of the kidney transplant recipients.

HLA genotyping. Recipient and donor HLA typing was performed according to the method of DNA PCR amplification and typing, using molecular sequence-specific priming methods for low-resolution typing for the HLA-A, -B, -C, -DRB1 and -DQB1 genetic loci (cat. nos. LTPPSD2, LTPPSD3, LTPPSD6, LTPPSD1, LTPPSD19; One Lambda, Inc). A combination of amplification primers, phosphorylated deoxynucleotides (dNTPs) and *Taq* DNA polymerase (total volume: 20 μl) were mixed with pre-coordinated DNA samples. After denaturation and neutralization, the material was first homogenized with a hybridization buffer and microbeads and subsequently labeled with Streptavidin, R-Phycoerythrin conjugate (SAPE; Thermo Fisher Scientific, Inc.) The fluorescence intensity of phycoerythrin in each microsphere was determined by reading the fluorescence signal with LABScan™ 100 (Luminex®; One Lambda, Inc.). The generated files were then imported into HLA fusion® software version 2.0 (One Lambda, Inc.) for analysis. PCR reactions were performed for 40 cycles, with denaturation at 93˚C for 30 sec, annealing at 65˚C for 30 sec and extension at 72˚C for 30 sec using a GeneAmp9700 thermal cycler (Applied Biosystems, Inc.).

Anti-HLA and anti-MICA screening. Patient serum was collected prior to transplantation. Anti-HLA and anti-MICA antibodies were subsequently detected using multiplexed microsphere-based flow cytometry (Luminex Technology; a LABScreen Mixed kit; One Lambda, Inc.). The microbeads were covered with the major HLA class I and II antigens and MICA antigens. Patients' sera were subsequently incubated for 30 min with beads covered with HLA antigens and MICA

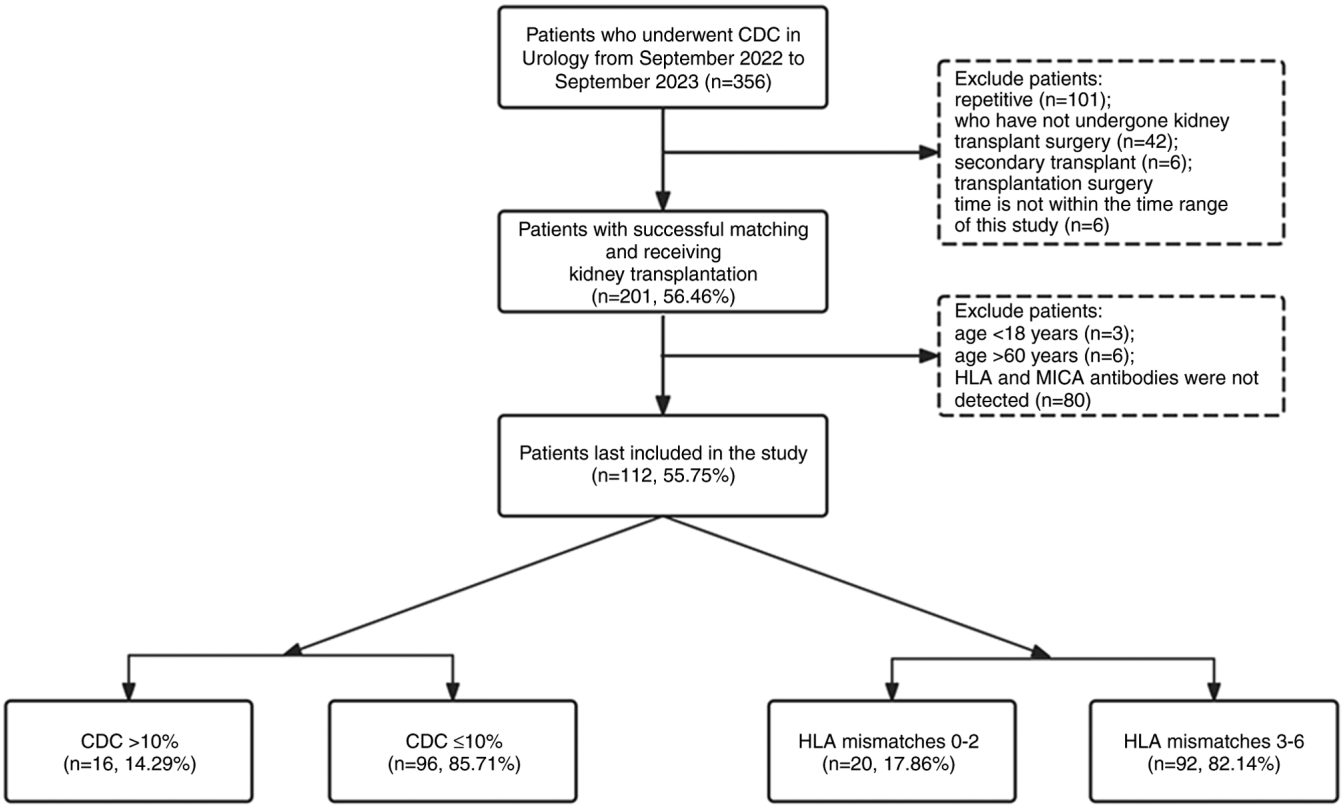

Figure 1. Flow chart of the design and study populations. A total of 356 kidney transplant recipients were screened and 112 patients eventually included in the analysis. CDC, complement-dependent cytotoxicity; HLA, human leukocyte antigen; MICA, major histocompatibility complex class I chain-related.

antigens produced by recombinant technology. After three washes, the beads were incubated for 30 min with 100 μl 1:100 phycoerythrin-labelled goat anti-human IgG (One Lambda, Inc.). After two further washes, the mean fluorescence intensity (MFI) of each microbead was measured using LABScan™ 100 flow cytometry (One Lambda, Inc.) and subsequently analyzed using HLA Fusion® software version 2.0 (One Lambda, Inc.). Samples with an MFI ≥500 were considered to be positive.

Induction protocol and maintenance immunosuppression. All included recipients were administered an oral triple immunosuppressive regimen comprising TAC, MMF and glucocorticoids starting on admission. The initial dose of TAC was 0.16 mg/kg/day orally, with a target trough level of 8-12 ng/ml for the first 3 months and 3-8 ng/ml thereafter. Start 0.5 g of MMF on the day of surgery and use 1 g per day postoperatively (0.5 g bid) and adjust as tolerated by the patient. High-dose methylprednisolone shock therapy was used in the early postoperative period, and the prednisone dose was reduced to 5-10 mg/day depending on the patient's condition. Anti-thymocyte globulin was used mainly in sensitized patients (defined as having CDC >10% or testing positive for panel-reactive antibodies), or in patients with a high number of HLA mismatches. Sulfamethoxazole and ganciclovir were used as antimicrobial prophylaxis drugs and the majority of the patients routinely underwent pre-operative hemodialysis or peritoneal dialysis to remove toxins from the body. Prior to transplantation, ABO-incompatible (ABOi) recipients received immunosuppressive preconditioning for 1-4 weeks, with different preconditioning regimens selected according to the recipient's initial anti-A/-B antibody titer. All ABOi renal transplant recipients were administered rituximab at a dosage of between 200-500 mg ~2 weeks prior to surgery. Recipients with an initial anti-A/-B antibody titer ≤1:4 were administered rituximab only, whereas those with an initial anti-A/-B antibody titer ≥1:8 were additionally treated with plasmapheresis and/or double-filtration plasmapheresis, as appropriate, to remove existing anti-A/-B antibodies. ABOi-associated living kidney transplantation could be performed after the anti-A/-B antibody titer was reduced to ≤1:8.

Data collection and outcomes. Clinical and laboratory examination data were collected from medical records and kidney transplant program databases at pre-transplantation, 1, 2, 3 and 7-day, 1, 3 and 6-month post-transplantation stages. Laboratory examination data included the following: The concentrations of serum creatinine (Scr) and urea, estimated glomerular filtration rate (eGFR), the levels of blood glucose (GLU) and hemoglobin (Hb), percentages of reticulocytes (RET%), absolute reticulocyte number (RET#), reticulocyte hemoglobin content (RET-He), low-fluorescence intensity reticulocytes (LFRs), medium-fluorescence intensity reticulocytes (MFRs), high-fluorescence intensity reticulocytes (HFRs), white blood cell count (WBC), the percentage of neutrophils (N%) and the absolute number of neutrophils (N#) and the percentage of lymphocytes (L%) and the absolute number of lymphocytes (L#) were measured. Renal function indicators were measured using a Roche automated biochemistry analyzer (Roche Corporation). Blood routine indexes were tested using a SYSMEX™ XE-2100 automatic five-category blood cell

analyzer. The occurrence of post-transplant adverse events was associated with DGF and DGF was defined either as having received dialysis within 1 week of transplantation or having an Scr >400 μmol/l within 7 days post-operatively despite not requiring hemodialysis.

Statistical analysis. Continuous variables are presented as the mean ± standard deviation or as the median and the interquartile range and categorical data are expressed as the number (%). Categorical data, including demographic, clinical and immunological characteristics, were analyzed using Pearson's χ^2 test (or χ^2 for trend testing when three categories of variables were present) or Fisher's exact test, whereas continuous variables were analyzed using the t-test (or one-way ANOVA for three categories of variables) or the Mann-Whitney U test (Kruskal-Wallis test for three categories of variables), as appropriate. Post hoc analyses were conducted using the Bonferroni method. Odds ratios (ORs) and P-values for clinically relevant covariates were obtained by applying binary logistic regression in univariate analyses, taking into account variables such as HLA mismatches, the age and sex of the patients, CDC results, blood group ABOi, donor type and pre-transplant HLA antibodies. Variables with P<0.05 in the univariate analysis were introduced into the multivariate model and significant predictors of the occurrence of adverse events were identified by multivariate logistic regression analysis. Statistical analyses were performed using the SPSS 25.0 software package (IBM Corp.). P<0.05 was considered to indicate a statistically significant difference.

Results

Demographic and clinical characteristics of the patients. A total of 356 patients underwent kidney transplantation between June 2022 and June 2023. Patients who did not meet the age requirement, were not first-time transplant recipients, or had missing antibody screening data (see the *Patients and methods* section) were excluded from the present study, resulting in the inclusion of a total of 112 patients in the analysis. Among them, 84 (75%) were male and 28 (25%) were female, with an average age of 38.17±9.08 years. The baseline and clinical characteristics of the patients included in the present study are shown in Table I.

When patients were stratified according to CDC-XM levels, the CDC >10% group consisted of all male patients, mainly with HLA-DR and -DQ MM and the majority of them had two alleles mismatched. Only one patient was found to have anti-HLA and anti-MICA antibodies prior to transplantation, of which the HLA antibody was HLA class I-positive and class II-negative and the remaining 15 patients were not found to have anti-HLA or anti-MICA antibodies. Furthermore, none of the patients in this group were found to have class II anti-HLA antibodies prior to transplantation. Alternatively, when patients were stratified according to HLA matching, the donors in the HLA 0-2 MM group were all from living donors (LDs) and the patients in this group had a CDC ≤10%, whereas all patients with CDC >10% were in the HLA 3-6 MM group. Moreover, all patients in the HLA 0-2 MM group were found not to have class II anti-HLA antibodies prior to transplantation, whereas six patients in the HLA 3-6 MM group were found to have class II anti-HLA antibodies prior to transplantation, of which two patients had antibodies against HLA-DQ and the other four patients had antibodies against HLA-DR. No DGF was identified in the HLA 0-2 MM group, which was significantly different compared with that in the HLA 3-6 MM group (P=0.022). In the CDC ≤10% and HLA 3-6 MM groups, almost half of the donor kidneys came from LDs (55.2 and 42.4%, respectively) whereas half of them came from donation after cardiac death (DCD) (44.8 and 57.6%, respectively).

Of all patients, the vast majority were ABO-compatible (ABOc), with only six (5.3%) patients being ABOi. A total of twenty-eight (25%) of the patients had anti-HLA and/or anti-MICA antibodies prior to transplantation, of which 15 (13.4%) patients were only anti-HLA antibody-positive, seven (6.3%) patients were only anti-MICA antibody-positive and six (5.3%) patients were positive for both antibodies. Moreover, 15 (13.4%) patients were only anti-HLA class I antibody-positive, three (2.7%) patients were only class II anti-HLA antibody-positive and three (2.7%) patients were positive for both antibodies. Finally, a total of 40 (35.7%) patients had anti-HLA and/or anti-MICA antibodies following transplantation, which represented an increase compared with the numbers of patients prior to transplantation.

Association between CDC-XM and laboratory results following kidney transplantation. Subsequently, the renal function of the two groups of patients with different CDC-XM levels were analyzed, which revealed that the levels of Scr decreased with post-operative time, leveling off after 7 days (Fig. 2A). The urea levels were found to decrease with time following surgery, leveling off after 1 month and a significant difference in the urea levels was observed between the two groups at 3 days post-operatively (P<0.05; Fig. 2B). By contrast, eGFR levels increased with post-operative time, leveling off after 7 days (Fig. 2C). The Scr levels of the CDC >10% group were found to be significantly higher compared with those of the CDC ≤10% group at 1 day (P=0.008), 2 days (P=0.006), 3 days (P=0.003) and 7 days (P=0.038) post-surgery (Table SIA). As for the eGFR levels, significantly higher eGFR levels were observed in the CDC ≤10% group at 1 day (P=0.038), 2 days (P=0.018) and 3 days (P=0.010) post-operatively (Fig. 2C and Table SIC).

The blood GLU levels in the two groups with different CDC-XM levels were maintained at high levels during the first 3 post-operative days, which was probably due to the influence of post-operative infusion. However, the blood GLU levels did not differ markedly between the two groups at all time points observed (Fig. 2D).

Subsequently, various indicators of erythrocytes in the two groups of patients with different CDC-XM levels were analyzed and the results showed that there was a statistically significant difference in the RET% and LFR values comparing between the two groups at 6 months (P<0.05; Fig. 2F and H), whereas no statistically significant differences in the Hb level or the RET#, MFR and HFR values, or the RET-He values were observed at all observed time points (P>0.05; Fig. 2 and Table SII).

Among the leukocyte indices, significant differences in the N%, L% and L# values were noted between the two groups at different CDC-XM levels (P<0.05; Fig. 2M, O and P), although no significant differences in the WBC and N# values were

Table I. Demographic and clinical characteristics of the study cohort.

Characteristic	CDC>10% n=16	CDC≤10% n=96	P-value	HLA 0-2 MM n=20	HLA 3-6 MM n=92	P-value
Age						
18-34 n (%)	7 (43.8)	39 (40.6)	1.000	9 (45.0)	37 (40.2)	0.502
35-49 n (%)	7 (43.8)	43 (44.8)		10 (50.0)	40 (43.5)	
50-60 n (%)	2 (12.5)	14 (14.6)		1 (5.0)	15 (16.93)	
Mean	38.17±9.08			38.17±9.08		
Sex (%)						
Male	16 (100)	68 (70.8)	0.029	12 (60.0)	72 (7 8.3)	0.087
Female	0 (0)	28 (29.2)		8 (40.0)	20 (21.7)	
Blood type compatibility, n (%)						
ABO-compatible	14 (87.5)	92 (95.8)	0.441	18 (90.0)	88 (95.7)	0.291
ABO-incompatible	2 (12.5)	4 (4.2)		2 (10.0)	4 (4.3)	
Donor category, n (%)						
LD	6 (37.5)	53 (55.2)	0.189	20 (100)	39 (42.4)	0.000
DCD	10 (62.5)	43 (44.8)		0 (0)	53 (57.6)	
HLA-A mismatches (%)						
0	0 (0)	12 (12.5)	0.219	10 (50)	2 (2.2)	0.000
1	10 (62.5)	62 (64.6)		10 (50)	62 (67.4)	
2	6 (37.5)	22 (22.9)		0 (0)	28 (30.4)	
HLA-B mismatches, n (%)						
0	1 (6.3)	6 (6.3)	0.626	6 (30)	1 (1.1)	0.000
1	6 (37.5)	47 (49.0)		14 (70)	39 (42.4)	
2	9 (56.3)	43 (44.8)		0 (0)	52 (56.5)	
HLA-C mismatches, n (%)						
0	1 (6.3)	11 (11.5)	0.718	7 (35)	5 (5.4)	0.000
1	9 (56.3)	58 (60.4)		13 (65)	54 (58.7)	
2	6 (37.5)	27 (28.1)		0 (0)	33 (35.9)	
HLA-DR mismatches, n (%)						
0	0 (0)	11 (11.5)	0.018	11 (55)	0 (0)	0.000
1	6 (37.5)	59 (61.5)		9 (45)	56 (60.9)	
2	10 (62.5)	26 (27.1)		0 (0)	36 (39.1)	
HLA-DQ mismatches, n (%)						
0	0 (0)	10 (10.4)	0.017	7 (35)	3 (3.3)	0.000
1	8 (50)	69 (71.9)		13 (65)	64 (69.6)	
2	8 (50)	17 (17.7)		0 (0)	25 (27.2)	
CDC, n (%)			-			
CDC>10%	-	-		0 (0)	16 (17.4)	0.071
CDC≤10%	-	-		20 (100)	76 (82.6)	
Pre-transplant serum antibodies, n (%)						
HLA+MICA+	1 (6.3)	5 (5.2)	0.219	1 (5.0)	5 (5.4)	0.321
HLA-MICA+	0 (0)	7 (7.3)		3 (15.0)	4 (4.3)	
HLA+MICA-	0 (0)	15 (15.6)		2 (10.0)	13 (14.1)	
MICA-HLA-	15 (93.8)	69 (71.9)		14 (70.0)	70 (76.1)	
Pre-transplant HLA antibodies, n (%)						
HLA class I+HLA class II+	0 (0)	3 (3.1)	0.789	0 (0)	3 (3.3)	1.000
HLA class I-HLA class II+	0 (0)	3 (3.1)		0 (0)	3 (3.3)	
HLA class I+HLA class II-	1 (6.3)	14 (14.6)		3 (15.0)	12 (13.0)	
HLA class I-HLA class II-	15 (93.8)	76 (79.2)		17 (85.0)	74 (80.4)	
Post-transplant serum antibodies, n (%)						
HLA+MICA+	1 (6.3)	5 (5.2)	1.000	2 (10.0)	4 (4.3)	0.567

Table I. Continued.

Characteristic	CDC>10% n=16	CDC≤10% n=96	P-value	HLA 0-2 MM n=20	HLA 3-6 MM n=92	P-value
HLA-MICA+	0 (0)	4 (4.2)		1 (5.0)	3 (3.3)	
HLA+MICA-	4 (25.0)	26 (27.1)		5 (25.0)	25 (27.2)	
MICA-HLA-	11 (68.8)	61 (63.5)		12 (60.0)	60 (65.2)	
Post-transplant HLA antibodies (%)						
HLA class I+HLA class II+	1 (6.3)	6 (6.3)	1.000	0 (0)	7 (7.6)	0.498
HLA class I-HLA class II+	1 (6.3)	5 (5.2)		1 (5.0)	5 (5.4)	
HLA class I+HLA class II-	3 (18.8)	20 (20.8)		6 (30.0)	17 (18.5)	
HLA class I-HLA class II-	11 (68.8)	65 (67.7)		13 (65.0)	63 (68.5)	
Pre-transplant antibody MFI median (IQR)						
Anti-HLA class I	0 (0-0)	0 (0-0)	0.234	0 (0-0)	0 (0-0)	0.957
Anti-HLA class II	0 (0-0)	0 (0-0)	0.306	0 (0-0)	0 (0-0)	0.243
Anti-MICA	0 (0-0)	0 (0-0)	0.530	0 (0-0)	0 (0-0)	0.199
Post-transplant antibody MFI median (IQR)						
Anti-HLA class I	0 (0-92.48)	0 (0-223.60)	0.281	0 (0-0)	0 (0-0)	0.073
Anti-HLA class II	0 (0-0)	0 (0-0)	0.715	0 (0-0)	0 (0-0)	0.076
Anti-MICA	0 (0-0)	0 (0-0)	0.141	0 (0-0)	0 (0-0)	0.147
Post-transplant de novo antibody, n (%)						
De novo HLA antibody	5 (31.3)	21 (21.9)75	0.522	4 (20.0)	22 (23.9)	0.924
De novo MICA antibody	0 (0)	4 (4.2)	1.000	0 (0)	4 (4.3)	1.000
Adverse event (within six months), n (%)						
DGF	2 (12.5)	17 (17.7)	0.877	0 (0)	19 (20.7)	0.022

CDC, complement-dependent cytotoxicity; MM, mismatch; LD, living donor; DCD, donation after cardiac death; HLA, human leukocyte antigen; MICA, major histocompatibility complex class I chain-related; MFI, median fluorescence intensity value; IQR, interquartile range; DGF, delayed graft function.

observed between the two groups (P>0.05; Fig. 2L and N). The N% values of the two groups were found to be significantly different at 3 days post-operatively (P=0.027; Table SIL). Similarly, significant differences in the L% values between the two groups were noted at pre-transplant (P=0.031), 2 days (P=0.007), 3 days (P=0.002) and 7 days (P=0.040) post-operative kidney transplantation (Table SIN). Moreover, a significant difference in L# values was observed between the two groups at 2 days (P=0.020), 3 days (P=0.013) and 7 days (P=0.015) post-operatively (Table SIO). Furthermore, the values of L% and L# decreased sharply at 1 day post-operatively and remained at extremely low levels for the first 3 days following transplantation, before beginning to gradually increase, eventually returning to pre-operative levels and leveling off at 1 month (Fig. 2O and P).

Association between HLA compatibility and laboratory results following kidney transplantation. Subsequently, patients were grouped according to the degree of HLA matching and the curve changes of the renal function indicators Scr, urea and eGFR were found to be similar to those in the CDC groups. Kidney transplant recipients in the HLA 0-2 MM group had significantly lower Scr levels compared with those in the HLA 3-6 MM group at 1 day (P<0.001), 2 days (P<0.001), 3 days (P<0.001), 7 days (P<0.001) and 1 month (P=0.041) post-operatively. Although a slight trend was observed at the pre-transplant stage, this did not reach the level of significance (P=0.096; Table SIIIA). Regarding the urea levels, significantly higher values were observed in the HLA 3-6 MM group at 2 days (P<0.001), 3 days (P<0.001) and 7 days (P=0.001) (Fig. 3B and Table SIIIB) compared with the HLA 0-2 MM group. At 1 day (P=0.001), 2 days (P<0.001), 3 days (P<0.001) and 7 days (P<0.001) following renal transplantation, the eGFR levels were significantly higher in the HLA 0-2 MM group (Fig. 3C; Table SIIIC).

Blood GLU level in the HLA 0-2 MM group was significantly lower compared with that in the HLA 3-6 MM group at the pre-transplant (P=0.002), 1-day (P=0.002), 2-day (P=0.021), 7-day (P=0.019), 1-month (P=0.038) and 3-month (P=0.030) stages. In addition, the HLA 0-2 MM group was found to have lower blood GLU levels compared with the HLA 3-6 MM group during the observation period (Fig. 3D and Table SIIID).

Regarding the various indicators of erythrocytes that were monitored in the present study, including Hb levels, RET%, RET#, LFR, MFR and HFR values and the RET-He, no significant differences were identified between the two groups at all pre-operative and post-operative time points (P>0.05; Tables SIV and SIIIE-J).

Subsequently, the various leukocyte indices between the HLA 0-2 MM and HLA 3-6 MM groups were compared,

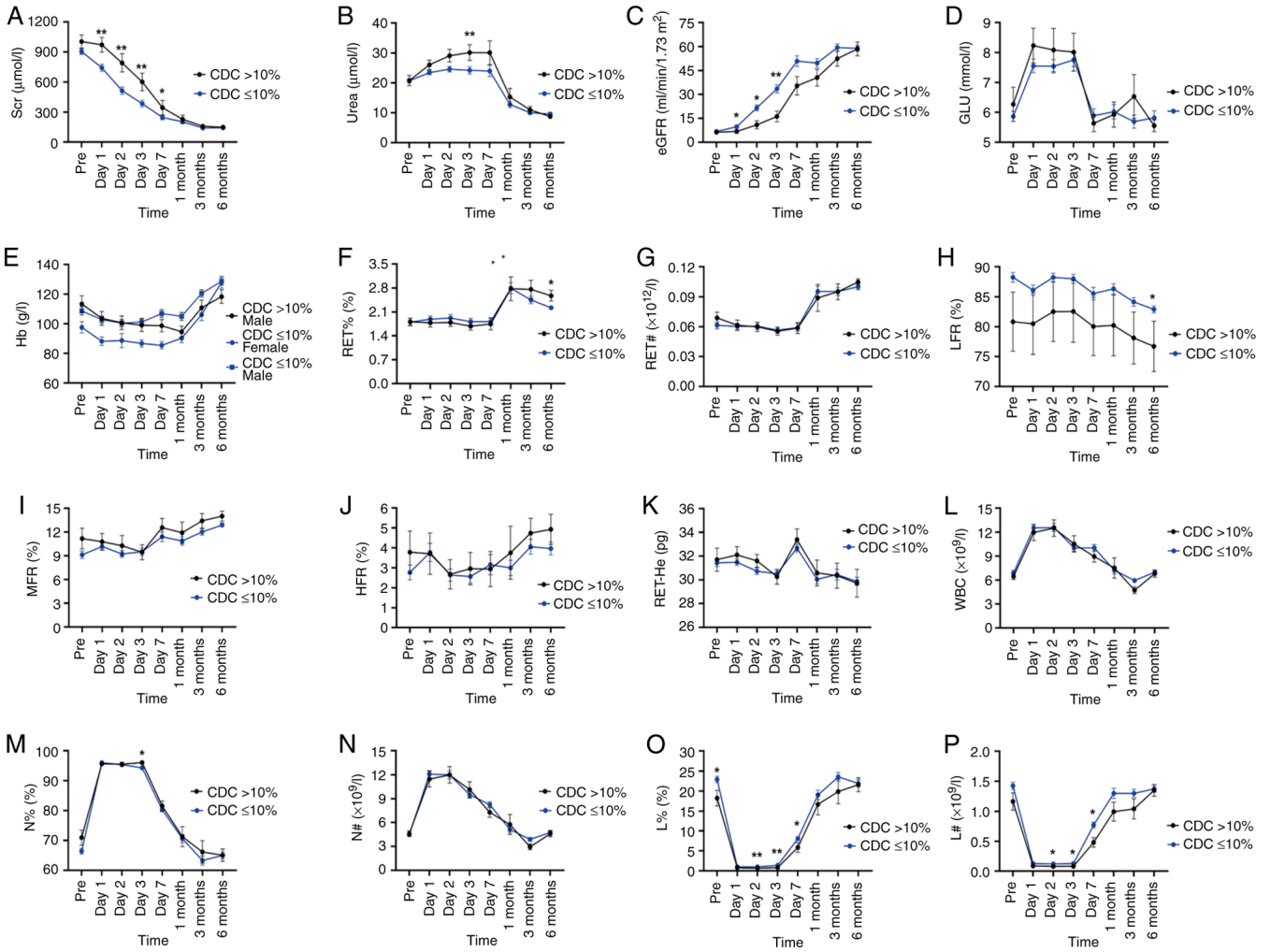

Figure 2. Laboratory indicator level of renal transplant recipients according to the CDC-XM levels, which represented in a line graph. (A) Dynamics of Scr at each follow-up time point. (B) Dynamics of urea at each follow-up time point. (C) Dynamics of eGFR at each follow-up time point. (D) Dynamics of GLU at each follow-up time point. (E) Dynamics of Hb at each follow-up time point. (F) Dynamics of RET% at each follow-up time point. (G) Dynamics of RET# at each follow-up time point. (H) Dynamics of LFR at each follow-up time point. (I) Dynamics of MFR at each follow-up time point. (J) Dynamics of HFR at each follow-up time point. (K) Dynamics of RET-He at each follow-up time point. (L) Dynamics of WBC at each follow-up time point. (M) Dynamics of N% at each follow-up time point. (N) Dynamics of N# at each follow-up time point. (O) Dynamics of L% at each follow-up time point. (P) Dynamics of L# at each follow-up time point. *P≤0.05 and **P≤0.01. RTR Scr, serum creatinine; eGFR, estimated glomerular filtration rate; GLU, blood glucose; Hb, hemoglobin; RET%, percentages of reticulocytes; RET#, absolute reticulocyte number; RET-He, reticulocyte hemoglobin content; LFR, low-fluorescence intensity reticulocytes; MFR, medium-fluorescence intensity reticulocytes; HFR, high-fluorescence intensity reticulocytes; WBC, white blood cell count; N%, the percentage of neutrophils; N#, the absolute number of neutrophils; L%, the percentage of lymphocytes; L#, the absolute number of lymphocytes; SEM, standard error of mean.

which revealed that the differences in WBC, N# and L% values were not statistically significant (P>0.05; Fig. 3L, N and O), although the differences in the N% and L# values between the two groups were statistically significant (P<0.05; Fig. 3M and P). The N% values were significantly lower in the HLA 0-2 MM group compared with the HLA 3-6 MM group at 7 days (P=0.017; Table SIIIL) post-operatively. Regarding the L# levels, significantly higher L# values were observed in the HLA 0-2 MM group at 7 days (P=0.030), 1 month (P=0.041) and 6 months (P=0.012) post-operatively (Fig. 3P and Table SIIIO). Finally, the curves of the L% and L# values were found to be similar to those of the CDC groups (Fig. 3O and P).

Effects of CDC-XM and HLA compatibility on renal function. When the CDC levels and HLA matching were assessed together, all patients were divided into three groups: Group 1 (CDC >10% and HLA 3-6 MM), group 2a (CDC ≤10% and HLA 0-2 MM) and group 2b (CDC ≤10% and HLA 3-6 MM). This analysis revealed that patients in group 2a (that is, who met both CDC ≤10% and HLA 0-2 MM) had improved results compared with both groups 1 and 2b in terms of their Scr, urea and eGFR data at all follow-up time points following surgery and also markedly faster and improved recovery levels of renal function within one week following surgery (Fig. 4 and Table SV). Urinary protein (qualitative) data in routine urinalysis, which showed significant differences only in the HLA subgroups at the second postoperative month (Fig. S1; Tables SIX and SX.).

Effects of HLA-A, -B and -DR mismatches on renal function. Subsequently, the impact of mismatches at each locus

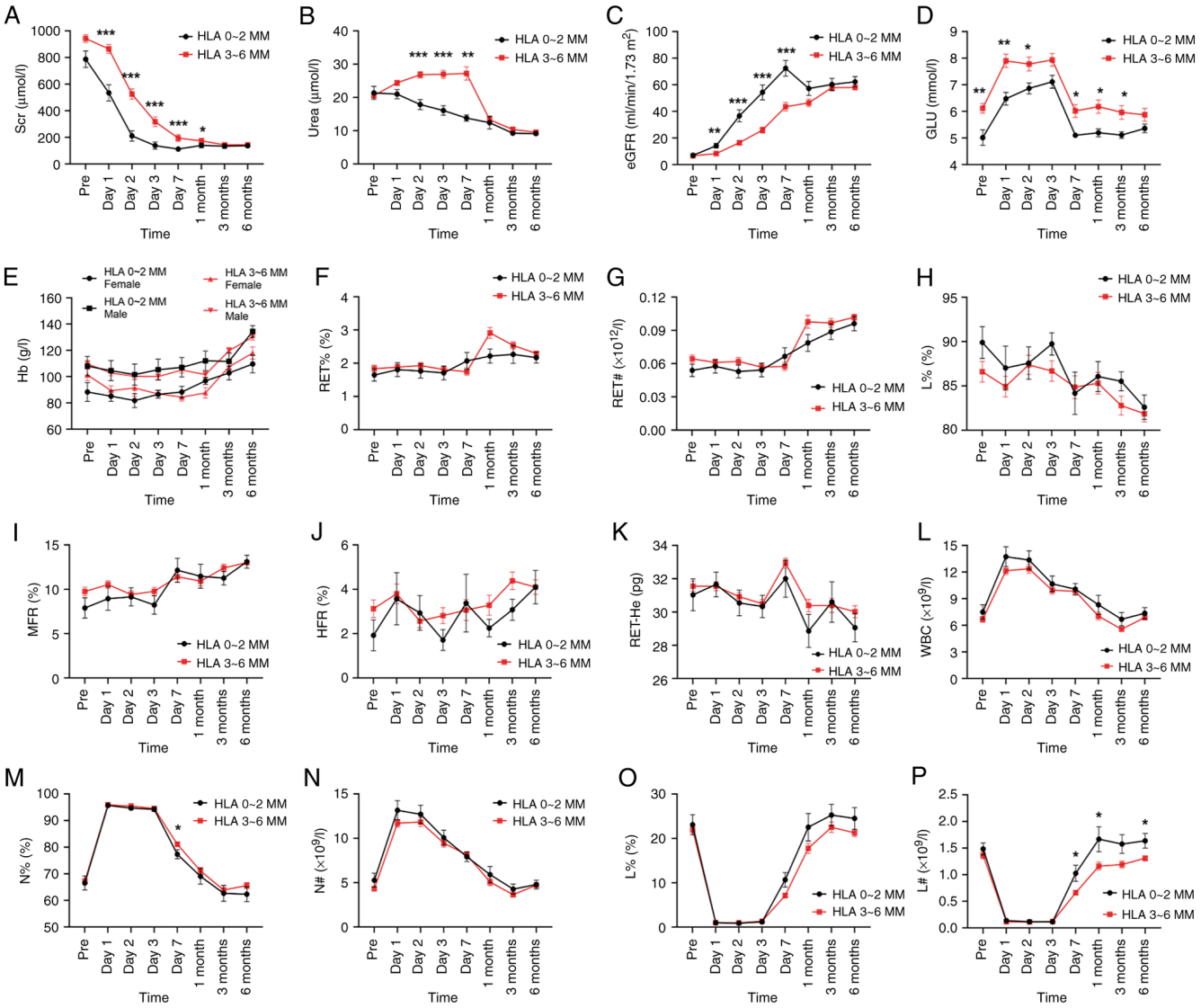

Figure 3. Laboratory indicator level of renal transplant recipients according to the HLA matching, which represented in a line graph. (A) Dynamics of Scr at each follow-up time point. (B) Dynamics of urea at each follow-up time point. (C) Dynamics of eGFR at each follow-up time point. (D) Dynamics of GLU at each follow-up time point. (E) Dynamics of Hb at each follow-up time point. (F) Dynamics of RET% at each follow-up time point. (G) Dynamics of RET# at each follow-up time point. (H) Dynamics of LFR at each follow-up time point. (I) Dynamics of MFR at each follow-up time point. (J) Dynamics of HFR at each follow-up time point. (K) Dynamics of RET-He at each follow-up time point. (L) Dynamics of WBC at each follow-up time point. (M) Dynamics of N% at each follow-up time point. (N) Dynamics of N# at each follow-up time point. (O) Dynamics of L% at each follow-up time point. (P) Dynamics of L# at each follow-up time point. Values are represented as the mean ± SEM. Values of $P \leq 0.05$ were considered statistically significant. $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$. Scr, serum creatinine; eGFR, estimated glomerular filtration rate; GLU, blood glucose; Hb, hemoglobin; RET%, percentages of reticulocytes; RET#, absolute reticulocyte number; RET-He, reticulocyte hemoglobin content; LFR, low-fluorescence intensity reticulocytes; MFR, medium-fluorescence intensity reticulocytes; HFR, high-fluorescence intensity reticulocytes; WBC, white blood cell count; N%, the percentage of neutrophils; N#, the absolute number of neutrophils; L%, the percentage of lymphocytes; L#, the absolute number of lymphocytes.

of HLA-A, HLA-B and HLA-DR on post-operative renal function was evaluated and this analysis revealed that patients who received 0-allele MM or 1-allele MM kidney transplants had an improved renal function compared with those who received a 2-allele MM transplant; moreover, the matching effect was very significant at the A locus comparing among the three loci and these patients experienced a greater impact on kidney function compared with the B and DR loci (Tables SVI-SVIII). In addition, the Scr, urea and eGFR values were found to be markedly improved in patients who received 0-allele MM or 1-allele MM kidney transplants compared with those who received a 2-allele MM kidney transplant at each follow-up time point one week after transplantation (Table SVI). However, in the patients with MM at the B and DR loci, the differences in renal function were mainly observed comparing between the 1-allele and 2-allele MM groups. Although renal function was optimal in patients with 0-allele MM in the three locus MM groups, no significant differences were observed at each follow-up time point following transplantation comparing between 0-allele MM and 1-allele MM transplanted patients and this was probably due to the small number of patients studied in the 0-allele MM group. There was no significant difference in renal function between ABO-compatible and -incompatible recipients (Fig. S2 and Table SXI). However, in the early postoperative period, recipients receiving LD kidney

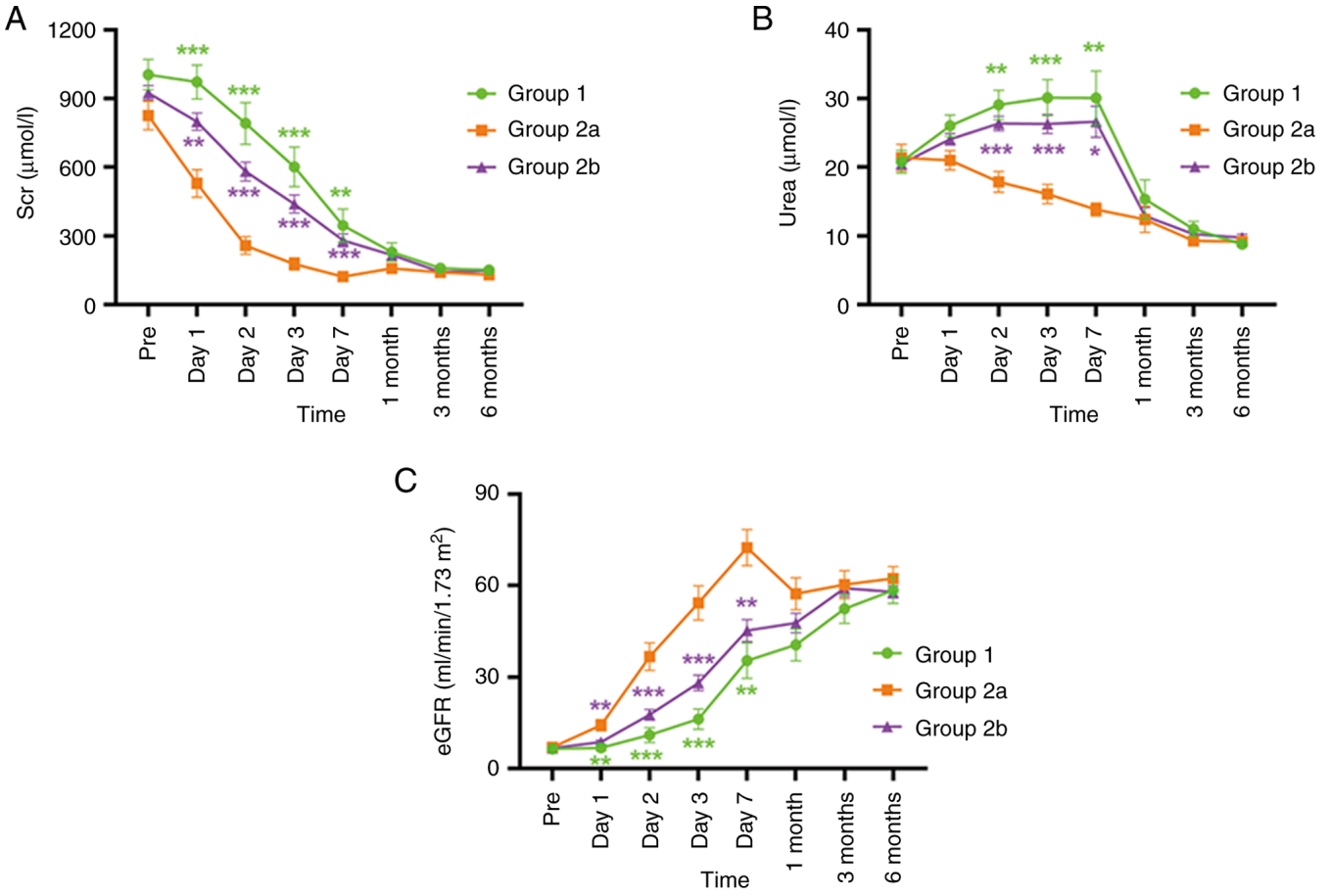

Figure 4. Renal function indicator level of renal transplant recipients according to the CDC-XM levels and HLA matching. (A) Serum creatinine, (B) urea and (C) estimated glomerular filtration levels of renal transplant recipients according to the CDC-XM levels and HLA matching. *P≤0.05; **P≤0.01; ***P≤0.001. The different colors of * represent the statistical results for that group vs. Group 2a. Scr, serum creatinine; eGFR, estimated glomerular filtration rate; SEM, standard error of mean; CDC-XM, complement-dependent cytotoxicity crossmatch; HLA, human leukocyte antigen.

transplantation had better renal function than recipients receiving DCD (Fig.S3 and Table SXII) .

Incidence of adverse events and multivariate logistic regression analysis. The incidence of DGF (20.7%) in the HLA 3-6 MM group was found to be higher compared with that in the HLA 0-2 MM group and this difference was statistically significant (P=0.022; Fig. 5A). However, this difference was not significant in the CDC-XM group (Fig. S4E). Subsequently, the proportion of patients with adverse events was analyzed in the MM patient groups with different HLA loci. The results obtained demonstrated that the highest proportion of patients with DGF was observed in the 2-B MM group (32.7%) and this difference was found to be statistically significant (Fig. 5B). No clear HLA matching effects of the HLA-C, -DR and -DQ loci were identified in the occurrence of adverse events; however, all of them were associated with an increase in the number of allelic mismatches and a corresponding increase in the incidence of adverse event rates, accordingly. Notably, no patients in the HLA 0-2 MM or the HLA-DR 0 MM groups developed DGF (Figs. 5A and S1C). In addition, the incidence of DFG was not significant in ABO-compatible compared with incompatible renal transplant recipients, and none of the six ABOi patients developed DGF (Fig. S5).

Of the 112 adult kidney transplant cases, 19 (17.0%) developed DGF (Table II). Patients who develop DGF were more likely to be male (47.4 vs. 20.4%; P=0.029) and older (45.3 vs. 36.7 years; P<0.001). Furthermore, they tended to have a higher BMI (24.06 vs. 22.4%; P=0.042), a poorer HLA match condition (3-6 HLA-A+B+DR mismatches: 100 vs. 78.5%; P=0.022) and diabetes mellitus as the underlying cause of ESRD (21.1 vs. 3.2%; P=0.016). Moreover, the ABOi (21.1 vs. 2.2; P=0.006) and DCD (94.7 vs. 38.7; P=0.000) values were also higher in patients with DGF compared with those without DGF. In the multivariable logistic regression analysis, recipients whose age was ≥40 years and who had a BMI ≥25, had the strongest predictors of DGF (ORs=1.097 and 1.555 and P=0.030 and 0.005, respectively; Table III). Finally, a reduced risk of DGF was identified in LD transplantation cases (OR=0.026; P=0.006), or in cases with a good HLA match (OR=0.378; P=0.029).

Discussion

It is well known that CDC-XM and HLA typing are two important methods to assess donor-recipient compatibility and for predicting the occurrence of transplant rejection. In the present study, the differences in laboratory results and clinical outcomes of kidney transplant recipients were evaluated

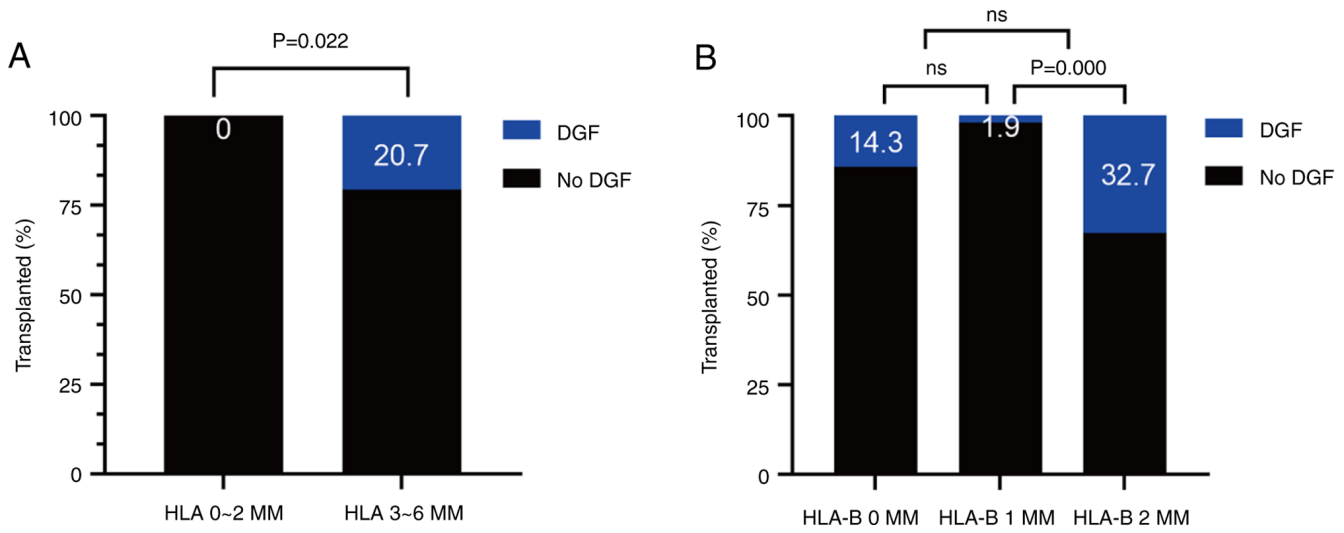

Figure 5. Comparison of the incidence of adverse events. (A) Comparison of the incidence of DGF with varying degrees of HLA matching; (B) Comparison of the incidence of DGF with different degrees of HLA-B locus matching. Values of P≤0.05 were considered statistically significant. ns, P>0.05; DGF, delayed graft function; HLA, human leukocyte antigen.

from the pre-operative stage to 6 months post-operatively, comparing between groups based on pre-transplantation CDC-XM levels and HLA-A+B+DR matching stratification, respectively. CDC-XM was the first method that was developed to assess donor-recipient compatibility and its implementation allowed transplants to be performed safely by mitigating the risk of hyper-ARs. However, the method has the disadvantages of lacking specificity and sensitivity. As a result, several modifications have been made to the standard CDC-XM assay over the years to improve its sensitivity, including the introduction of a washing step, extended incubation times and the addition of anti-human globulin reagents (6). In addition, treatment of the serum with dithiothreitol was introduced to distinguish genuine positive XM reactions from those triggered by clinically irrelevant IgM antibodies (7). More sensitive and specific HLA antibody detection techniques have been developed over the most recent three decades, including flow cytometry crossmatch (FCXM) methods and Luminex®-based single antigen beads (SAB) assay. Given the expansive development of these histocompatibility-testing methods that has occurred compared with CDC-XM, which showed poor sensitivity and high false-positive reactivity, FCXM and SAB have become more favored as tests for the pre-transplantation screening of organs. However, in numerous countries in Asia, CDC-XM is still retained due to its cost-effectiveness. By contrast, a vast majority of transplant centers in North America and also in several European countries such as the United Kingdom, have replaced CDC-XM with more sensitive FCXM techniques. To improve the accuracy of immunization risk assessment, laboratories have also seen the introduction of FCXM and SAB techniques. In clinical practice, patients with a CDC result of 11-20% may be eligible for kidney transplantation following a comprehensive evaluation. It should be noted that the success of kidney transplantation is also affected by numerous other factors, including other immune compatibility indicators of the donor and recipient, immunosuppressive therapy following transplantation, among other factors. Since the majority of studies to date have excluded patients with CDC >10% and relatively few studies have been published on the prognosis of kidney transplant patients with CDC >10%, these patients were included in the analysis in the present study and a comparative analysis was also performed with patients with CDC ≤10%.

As the number of HLA-A+B+DR mismatches increases, so does the risk of rejection after kidney transplantation and the likelihood of graft loss. In a comparative analysis of over 130,000 kidney transplant survival cases, a statistically strong and significant HLA matching effect was shown when HLA-A+B+DR mismatching was used as a reference (8). Therefore, HLA-A+B+DR locus matching is considered to be the central cornerstone of renal allocation. When HLA-A+B+DR mismatching involves 0, 1 or 2 alleles, a high degree of donor-recipient matching may still be achieved, although the number of HLA antigens, the number of antigen combinations and their unequal distribution across racial and ethnic populations makes it very difficult to find well-matched donors, especially in areas with a diverse population (9). Of the 112 patients in the present study, only 20 (17.9%) patients were well-matched; that is, less than one-fifth of the total study population. In the past, the effect of HLA-DQ matching has been underemphasized and DQ antigens have not yet been part of kidney allocation algorithms due to the high association of DR antigens with certain DQ antigens, a phenomenon known as 'linkage disequilibrium'. However, there is growing evidence that HLA-DQ mismatching can act as a predictor of AR and that it is independent of multiple relevant clinical covariates (10,11). In the present study, the incidence of DGF in the 2-DQ MM group (32%) was only 0.7% lower compared with that in the group with the highest incidence of DGF (namely, the 2-B MM group; 32.7%) and the incidence was higher compared with that in the 2-A MM (17%) and 2-DR MM (25%) groups. Therefore, additional studies are required to further evaluate whether it is necessary to additionally quantify the effect of HLA-DQ matching compared with the HLA-A, -B and -DR loci. The value of HLA matching

Table II. Demographics of study patients.

Characteristic	No DGF n=93	DGF n=19	P-value
Mean age, years	36.7±8.4	45.3±8.8	0.000
Sex, n (%)			0.029
Male	74 (79.6)	10 (52.6)	
Female	19 (20.4)	9 (47.4)	
Mean BMI (kg/m^2)	22.4±3.2	24.06±2.6	0.042
Diabetes, n (%)	3 (3.2)	4 (21.1)	0.016
CDC, n (%)			0.704
CDC>10%	14 (17.2)	2 (10.5)	
CDC≤10%	79 (82.8)	17 (89.5)	
Donor category, n (%)			0.000
LD	57 (61.3)	1 (5.3)	
DCD	36 (38.7)	18 (94.7)	
Pre-transplant HLA antibodies, n (%)			0.720
HLA class I+HLA class II+	2 (2.2)	0 (0)	
HLA class I-HLA class II+	2 (2.2)	1 (5.3)	
HLA class I+HLA class II-	12 (12.9)	3 (15.8)	
HLA class I-HLA class II-	77 (82.7)	15 (78.9)	
Mean HLA-A+B+DR mismatches			0.022
0-2	20 (21.5)	0 (0)	
3-6	73 (78.5)	19 (100)	
Types of dialysis, n (%)			0.516
HD	58 (62.3)	15 (78.9)	
PD	23 (24.7)	4 (21.1)	
Mean time on dialysis, years	2.9±4.1	3.5±4.0	0.074
ABO incompatibility, n (%)	2 (2.2)	4 (21.1)	0.006

DGF, delayed graft function; BMI, body mass index; CDC, complement-dependent cytotoxicity; HD, Hemodialysis; PD, Peritoneal dialysis; MM, mismatch; LD, living donor; DCD, donation after cardiac death; HLA, human leukocyte antigen; MICA, major histocompatibility complex class I chain-related; MFI, median fluorescence intensity value; IQR, interquartile range.

Table III. Multivariate regression analysis of DGF.

Variable	OR	95%CI	P-value
Recipient age ≥40	1.097	1.009-1.194	0.030
Recipient BMI ≥25	1.555	1.139-2.124	0.005
Recipient diabetes	0.147	0.010-2.117	0.159
Recipient sex	0.529	0.134-2.082	0.362
Donor category	0.026	0.002-0.346	0.006
Time on dialysis ≥5	0.883	0.762-1.023	0.097
ABO incompatibility	2.328	0.201-26.945	0.499
HLA-A+B+DR mismatches ≥3	0.378	0.158-0.903	0.029

OR, odds ratio; CI, confidence interval; BMI, body mass index; HLA, human leukocyte antigen.

in kidney transplantation and its role in prolonging graft survival, have been widely recognized. As a result, most organ-sharing organizations give preference to well-matched kidney recipients for transplantation. At present, HLA-typing techniques have evolved from simple serological methods to advanced molecular techniques that increase the importance of HLA matching by extending it to more precise matching at the allele (or even the epitope) level, which would minimize the risk of sensitization and thereby improve the success rate of transplantation.

The recently introduced solid-phase assay, Luminex®, provides a reliable and sensitive method for identifying panel-reactive antibodies and distinguishing between HLA class I and II antibodies. A study by Süsal *et al* (12) found that kidney transplant recipients who were negative for both HLA class I and II antibodies exhibited a higher 2-year graft survival rate compared with those who were positive for both HLA class I and II antibodies and this difference was found to be statistically significant (P<0.001). Similarly, Michielsen *et al* (13) reported a similar observation by analyzing the clinical correlation between pre-transplant DSA and non-donor-specific anti-HLA antibodies (nDSA). In the present study, no patients in the CDC >10% group were identified as being positive for both HLA-class I and II antibodies prior to transplantation. Also note that the sensitization degree of renal recipients with CDC >10% was higher compared with that of renal recipients with CDC ≤10%. Therefore, in the comprehensive evaluation of kidney transplantation for those patients with CDC >10%, attempts should be made to try to avoid selecting patients with positive pre-transplant HLA class I and II antibodies. Solgi *et al* (14) observed that pre-transplant sensitivity to HLA class II antigens was strongly associated with a higher incidence of AR during the first post-operative year (P=0.004). In the present study, the proportion of patients with post-operative adverse events decreased with increasing CDC levels, although the difference was not found to be significant. All patients in the CDC >10% group were negative for pre-transplant HLA class II antibodies, suggesting that negative pre-transplant HLA class II antibodies may be associated with improved clinical outcomes.

To address the insufficient numbers of kidney donors to meet clinical needs, efforts are being made worldwide to expand the donor pool, including performing kidney transplantations under CDC-positive and ABOi conditions. CDC-positivity is highly associated with hyper-AR and immediate graft loss and two major clinical desensitization methods, high-dose intravenous immunoglobulin (IVIG) and plasma exchange combined with low-dose IVIG, have been used to decrease the circulating anti-HLA antibody load in patients, thereby increasing the chance of transplantation for sensitized patients. ABOi has also long been considered a contraindication to successful transplantation. Increasingly critical organ supply shortages has forced the development of strategies to overcome the ABO antibody barrier. Desensitization is usually achieved using therapeutic apheresis and B-cell depletion therapies, which are accompanied by strong immunosuppression. Despite the use of stronger immunosuppressive therapies for ABOi kidney transplantation, no increases in the incidence of rejection, infectious complications or malignancy have been reported (15). In the present study, there were a total of six (5.3%) patients with ABOi, who received pre-operative desensitization until the anti-A/B antibody titer was below the target titer prior to transplantation. No significant difference in incidence of post-operative adverse events was observed between the ABOi and ABOc groups and the six patients with ABOi did not develop DGF post-operatively. ABOi living donor kidney transplant (LDKT) patients have significantly improved survival rates compared with patients who either remain on the waiting list (P=0.001) or who receive an ABOc deceased donor kidney transplant (DDKT; P=0.048) and that the higher the titer of anti-A/-B antibody in ABOi kidney transplant patients, the lower is the survival rate of the patients (16). In their study, Massie *et al* (17) also show that patients treated with ABOi-LDKT have higher cumulative survival rates at 5 and 10 years (90.0 and 75.4%, respectively) compared with similar patients who remained on the waiting list or received an ABOc-LDKT/ABOc-DDKT (81.9 and 68.4%, respectively). In the present study, levels of Scr, urea and eGFR did not differ markedly between the ABOi and ABOc groups at each follow-up time point during the observation period, which may have been associated with the intensive immunosuppressive therapy received by ABOi patients. Regarding the impact of long-term prognosis, in a study by others (18), similar renal function was found between these two types of patients; furthermore, no significant differences were identified in terms of the renal function comparing the two patient groups. In recent years, ABOi kidney transplantation has become a routine procedure. With this approach, ~30% of previously rejected LDs are now able to donate their kidneys, thereby markedly expanding the LD pool. However, transplantation in the presence of severe ABOi puts the patient at a higher risk of early rejection, infection and infection-associated mortality. Therefore, when possible, ABOc donors should be preferred for kidney transplantation.

Renal function monitoring may be used to assess the functional status of the transplanted kidney to determine whether the excretory function of the transplanted kidney is normal and whether there are problems such as AR or chronic rejection. In the present study, statistically significant differences were identified in terms of the Scr, urea and eGFR values in both groupings. During the observation period, especially within the first month after surgery, the renal function of the CDC ≤10% and HLA 0-2 MM groups was markedly improved compared with that of the CDC >10% and HLA 3-6 MM groups and the improved status of the performance of renal function was even more obvious when patients met the conditions of CDC ≤10% and HLA 0-2MM at the same time. This suggested that, in the early stage following kidney transplantation, CDC-negativity and improved HLA matching are more helpful in terms of facilitating the rapid and high-quality recovery of renal function in the short-term period after surgery. In the clinical setting, the measurement of Scr and urea nitrogen levels is of great value in terms of helping to determine renal function, as these fulfill a key role in maintaining metabolic homeostasis and eliminating metabolic wastes from the body: When both parameters are elevated, it is usually indicative of impaired renal function (19). Parameters such as urinary albumin and the urinary protein-to-creatinine ratio, which are used as markers of early renal impairment, may also be used to assess the extent of renal damage more accurately (20,21). However, the present study was unable to include such more sensitive indicators in the analysis due to the lack of quantitative tests for proteinuria and creatinine in their post-operative follow-up examinations. Although it did collect the results of qualitative tests for urinary proteins in the routine urinalysis, the present study found that the number of patients with positive urinary protein decreased over time during the 6 months following surgery and the proportions of patients with positive urinary protein in the CDC ≤10% and HLA 0-2 MM groups were smaller compared with those in the CDC >10% and HLA 3-6

MM groups at the same time point. However, clinical decision-making in renal disease depends heavily on eGFR levels, as this is the primary surrogate marker for clinical monitoring of allograft function and long-term graft survival (22,23). It has been reported previously that patients who receive kidney transplants with high eGFR levels that remain stable for one year tend to have transplanted organs from younger donors (23). These patients have a relatively low immunological risk and do not develop persistent allograft injury, as well as fewer histological lesions associated with allograft injury. The majority of the donors in the HLA 0-2 MM group in the present study were from LDs of similar age to the recipients and therefore all patients in this group had higher eGFR levels compared with those in the HLA 3-6 MM group post-operatively. A previous study of 325 DCD and 409 LD kidney transplantations revealed that eGFR was markedly lower in DCD renal recipients compared with LD renal recipients on post-operative days 7 and 30 (24). Similar results were found in the present study, where it was shown that kidney transplant recipients who received a transplant from an LD had markedly enhanced renal function, including the Scr, urea and eGFR levels, compared with kidney transplant recipients who received DCD at pre-transplant and each of the subsequent follow-up time points within one month post-operatively. Renal function was also markedly improved in the LD group compared with the DCD group at the pre-transplant stage due to the overall enhanced physical states of the LD group recipients compared with the DCD group. Furthermore, in addition to differences in renal function in the short-term post-operative period, other clinical outcomes, such as the graft survival rate, also differed between the donor types. Chen *et al* (25) analyzed 6,719 renal transplant recipients from the Chinese Renal Transplantation Scientific Registry and found that, compared with the transplants from DCD, improved outcomes were observed in the LD group, including the 3-year graft survival rate (LD=95.8% vs. DCD=91.3%), DGF (LD=2.4% vs. DCD=17.7%), infection (LD=10.7% vs. DCD=20.7%), graft loss (LD=2.3% vs. DCD=6.3%) and death (LD=1.3% vs. DCD=3.2%).

The erythrocyte system has been shown to be especially important for endocrine metabolism after renal transplantation. Erythropoietin (EPO) is a glycoprotein hormone produced mainly by the kidneys and its main effect is to stimulate erythropoiesis in the bone marrow, thereby increasing the concentration of Hb (26). It has been shown that testosterone induces an increase in Hb and hematocrit, an effect that has been associated with the stimulation of EPO expression and a decrease in the concentrations of ferritin and hepcidin (27). In the present study, the Hb levels of the same group of male patients were found to be higher compared with those of the female patients during the same time period, which may be due to the fact that the level of testosterone in males is higher compared with that of females and therefore the secretion of EPO is relatively higher, which consequently promotes erythropoiesis and Hb synthesis. Reticulocytes can effectively reflect the hematopoietic status of the body's erythrocyte system. In the present study, the majority of patients were mildly anemic prior to surgery, whereas in the middle and late post-operative periods, as the transplanted renal function gradually recovered, the secretion of EPO was observed to increase, the reticulocytes reached a higher level and the Hb level gradually increased. Compared with the pre-operative and early post-operative periods, the numbers of reticulocytes markedly increased, which led to a significant recovery of the patients' anemia. Iron deficiency (ID) is highly prevalent in kidney transplant recipients and has been shown to be independently associated with an excess mortality risk in this population (28). Moreover, the parameter RET-He is affected only by the amount of iron intake, but it accurately reflects iron deficiency in patients (29). RET-He of patients fluctuated consistently within the normal range in each group of patients, suggesting that iron deficiency was not present in these patients within the first 6 months following renal transplantation and that iron deficiency correction was not required. A previously published longitudinal study showed that patients with pre-transplant ID remained iron-deficient following transplantation and that ferritin levels tended to decrease in the first few months after transplantation (30). Therefore, the presence or absence of iron deficiency after transplantation depends on a variety of factors, including the patient's overall health, recovery of transplanted kidney function, dietary habits and the presence of chronic blood loss. In the time frame selected for the present study, in the CDC group, the RET% and LFR values were significantly different only at the 6-month post-operative stage ($P<0.05$). In the HLA group, the differences in Hb level, RET%, RET#, LFRs, MFRs, HFRs and RET-He were found not to be statistically significant ($P>0.05$) comparing between the two groups, suggesting that the pre-operative CDC level and HLA matching may not have a significant effect on post-operative erythropoiesis and metabolism, although this observation still needs to be confirmed by a retrospective or prospective study with a larger sample size.

Leukocytes form an important part of the human immune system and their primary functions include immune defense and participation in immune regulation. In particular, neutrophils and lymphocytes have critically important roles for transplant patients. As kidney transplantation is an invasive treatment modality, which in turn causes a stress response, the immune system exhibits a reactive inflammatory response, with the numbers of WBCs and neutrophils increasing rapidly from the first post-operative day to a level that is far above the normal range. In the present study, the patients' lymphocyte parameters were at extremely low levels within one week post-operatively and both the L# and L% values were markedly decreased compared with those of the pre-operative period. This may have been due to the use of immunosuppression (including lymphocyte depletion), as a direct reduction in the number of lymphocytes post-operatively has been noted in previous studies (31,32), or alternatively, the cause may have been a blockade of lymphocyte activation, conductance and expression (33). When the recovery in the number of lymphocytes started on the third post-operative day, the lymphocyte parameters L# and L% were consistently lower in the HLA 3-6 MM group compared with the HLA 0-2 MM group due to the persistence of stronger immunosuppression. Studies have also suggested that the neutrophil/lymphocyte ratio (NLR) and lymphocyte count may be risk factors for graft and patient prognosis. NLR is a simple tumor marker that predicts the prognosis of certain solid malignancies, including renal cell carcinoma, bladder uroepithelial carcinoma and prostate carcinoma (34,35). Another study shows that, when a patient's

lymphocyte count falls below 750/mm^3 at a given follow-up time, the risk of both graft failure and death is increased compared with similar patients who were without lymphopenia at the same time point (36). Therefore, neutrophil and lymphocyte parameters should be closely monitored during long-term follow-up following renal transplantation, as they are inexpensive to monitor, easily accessible and widely available parameters for assessing the prognostic status of renal transplantation. The present study did have certain limitations in that the examination of lymphocyte subsets was lacking in the routine follow-up of renal transplant patients in our hospital. However, over the past two decades, there has been increasing evidence to show that monitoring peripheral blood lymphocyte subsets in renal transplant patients is important for improving the success rate of renal transplantation, for protecting the function of the transplanted kidney and for improving the quality of life of patients (37-39).

DGF is a manifestation of post-transplant acute renal failure and is an important complication of kidney transplantation. In clinical series, the most frequently reported donor and recipient factors associated with DGF have been shown to be BMI, previous transplantation and diabetes in male recipients and increased age, donor type and BMI in female donors (40). Additional factors frequently reported are warm ischemia time, cold ischemia time, prior sensitization and HLA mismatches (41). The age and BMI of the recipient, as well as HLA mismatches, were confirmed in the present study as being important factors for the development of DGF. In the present study, the incidence of DGF was 1.7% in cases of LD kidney transplantation and 32.1% in cases of DCD kidney transplantation and these findings were found to be consistent with those of previous studies (42-44). None of the patients in the HLA 0-2 MM group developed DGF, probably since the donors in this group were all from LDs and had improved HLA matching. In addition to the non-immunological factors aforementioned, immune factors also have a role in the development of DGF. A serum study from a multicenter collaborative transplantation study shows that the presence of class I and II HLA antibodies prior to transplantation is a strong predictor that almost doubles the risk of DGF, whereas the presence of only class I HLA antibodies or only class II antibodies show no significant effect (45). Moreover, in another study, the risk of DGF was almost doubled in patients with pre-formed DSA compared with those either without HLA antibodies or with HLA antibodies against third-party antigens, despite the results of CDC-XM being negative (46). The aforementioned study also found that patients who developed antibodies directed against donor HLA-DRB1 had the highest incidence of DGF (69%), which may explain the phenomenon that no patients in the HLA-DR 0 MM group developed DGF in the present study. Due to the small sample size of the present study, however, which included 21 patients who were positive for HLA antibodies prior to transplantation and featured only three patients who were positive for both HLA class I and II antibodies, it was not possible to derive conclusions similar to those reported in other studies. At the same time, because few of the patients included in the present study had positive DSA, no large-sample size DSA data were available for analysis. In conclusion, it may be said that DGF has a significant negative impact on graft survival; therefore, it is crucial to take appropriate measures to prevent the development of DGF in renal transplant patients.

The present study did, however, also have a number of limitations. First, retrospective studies are limited by the availability of data and so it was not possible to include all the variables and indicators associated with the prognosis of renal transplant patients. Some of the examination indicators that are more relevant to disease and more specific are not available to us. For example, lymphocyte subsets, urinary albumin, or the ratio of urinary albumin to creatinine. Secondly, small amounts of data were missing and to compensate for this the present study employed appropriate algorithms to replace the missing data values, which may have led to biased results. Furthermore, since the follow-up period of the present study was from the pre-operative period to six months post-operatively, it was not possible to analyze the long-term outcomes of the patients. Additionally, the small sample size of the collected data and insufficient sample size of HLA 0-2MM group calculated by PASS software in HLA grouping may limit the general usability and reproducibility of the results of the present study. Therefore, prospective, multicenter trials with longer follow-up periods are needed to address the aforementioned issues, with a view to obtaining more comprehensive and reliable findings.

In conclusion, the present study has demonstrated that renal transplant recipients with CDC ≤10% and HLA 0-2 MM tended to have improved recovery of renal function in the early post-operative period compared with transplant recipients with CDC >10% and HLA 3-6 MM. When the conditions of CDC ≤10% and HLA 0-2 MM were both met, kidney transplant recipients had an improved post-operative prognosis, including faster recovery of renal function and lower incidence of DGF. In a multifactorial analysis of adverse events, recipient age, recipient BMI, donor type and HLA mismatch were significant risk factors for DGF. In addition, pre-operative CDC-XM levels and HLA matching did not markedly affect post-operative erythropoiesis or metabolism. Differences in laboratory findings between groups were mainly concentrated in the first week after the operation, whereas recovery between 1 and 6 months was essentially comparable, suggesting that the long-term recovery of kidney transplant recipients may be associated with a wider range of factors and that other factors including the source of the donor kidney, nDSA and DSA may also have an effect on the transplant results. Therefore, in kidney transplantation, doctors need to comprehensively consider various factors to formulate the optimal transplantation plan for patients.

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Availability of data and materials

The data generated in the present study are included in the figures and/or tables of this article.

Authors' contributions

MZ and WX conceived the present study. MZ, SX and CX participated in the design of the present study. SX, CX, ZZ, CQ, XS and XW participated in data collection. CX and ZZ analyzed and interpreted the data. CQ, XS and XW drafted the manuscript. SX, CX, ZZ, CQ, XS and XW revised and edited the manuscript. MZ, WX and SX confirmed the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The authors obtained appropriate institutional review board approval. Ethical approval was granted by The Ethics Committee of the First Affiliated Hospital of Anhui Medical University (approval no. PJ2024-05-30). The data and samples utilized in the present study were obtained from the hospital's electronic medical record system and did not involve any privacy or information of the patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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