# Relevance of Flow Cytometric Auto-Crossmatch to the Post-transplant Course of Kidney Transplant Recipients

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## ABSTRACT

**Introduction.** The crossmatch test is essential prior to kidney transplantation (tx) to confirm compatibility between the donor and the recipient. However, its results can be misleading due to "undetectable antibodies" in the recipient's serum. To establish if undetectable autoantibodies are responsible for a positive result, an auto-crossmatch test can be performed. In this study, we aim to determine the long-term prognostic value of auto-flow cytometric auto-crossmatch (FCXM) test on kidney survival in kidney tx recipients.

**Materials and Methods.** The primary outcome variable was reduced renal function. Secondary endpoints were incidence of biopsy-confirmed chronic antibody-mediated rejection (CAMR) and recurrent glomerulonephritis (GN).

**Results.** There were no differences regarding initial serum creatinine levels between the study and control groups ($P = .441$). Patients who had positive auto-B FCXM had a significantly reduced renal function compared with the control group ($P = .016$). Four patients developed biopsy-confirmed CAMR in the study group and 1 patient in the control group ($P = .047$). Five patients had biopsy-confirmed recurrent GN in the GN study group, and only 1 patient had recurrent GN in the GN control group ($P = .026$).

**Discussion.** Kidney transplant recipients with positive auto-FCXM test had significantly reduced renal function and a higher incidence of recurrent GN and CAMR compared with the control group. The findings of this study suggest a potential role of auto-antibody causing positive auto-FCXM test result, meanwhile increasing the risk of CAMR, recurrent GN, and new-onset diabetes after tx.

THE CROSSMATCH test is essential prior to kidney transplantation (tx) to confirm compatibility between the donor and the recipient, particularly to prevent hyperacute rejection. The classic lymphocyte complement-dependent cytotoxicity crossmatch (CDC-XM) has a high positive predictive value for graft loss (80%). Flow cytometric crossmatch (FCXM) is more sensitive than CDC-XM at detecting anti-HLA antibodies [1–3].

However, FCXM can be misleading due to false-positive results or "undetectable antibodies" in the recipient's serum. To establish if undetectable autoantibodies are responsible for the positive result, an auto-crossmatch test can be performed, where the recipient serum is crossmatched against his or her own lymphocytes [4,5]. However, clinical significance of these undetectable auto-antibodies has not been investigated before. In this study, we aim to determine the long-term prognostic value of auto-FCXM test on kidney survival in kidney tx recipients.

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## MATERIALS AND METHODS

### Patients

All patients 18 years of age and older who underwent kidney tx at the Istanbul Faculty of Medicine from October 1994 to October 2014 were included in the study. Patients who had positive auto-B-cell FCXM results and follow-up of more than 24 mounts following kidney tx were included in this analysis. Patients who had a positive CDC-XM or auto-T-cell FCXM test or who underwent an induction therapy before transplantation were excluded. All data of transplant recipients were recorded prospectively in a large database. The records of these patients were compared with a control group including living kidney tx recipients with negative auto-FCXM test.

The demographic, clinical, and biochemical data were collected from the medical records. Demographic and clinical data included age at kidney tx, gender, cause of end-stage renal disease, prior renal replacement treatment history, and post-tx follow-up. Causes of end-stage renal disease were classified into 3 groups: glomerulonephritis (GN), nonglomerular diseases, and other causes of kidney diseases. Hemoglobin level, white blood cell count, serum creatinine, blood urea nitrogen, glucose, albumin, calcium, phosphorus, triglyceride, high-density lipoprotein, low-density lipoprotein, and total cholesterol levels were measured every 3 months after kidney tx.

### Biopsy Assessment

Biopsies were based on the clinical course of the patients. Indications for biopsy were a significant increase of serum creatinine levels and/or de novo occurrence of proteinuria (>1 g/24 h). Biopsies were evaluated by the same pathologist. Kidney biopsy tissues from patients and the control group were processed for light microscopy and stained by immunofluorescence with conjugated antibodies to IgG, IgM, IgA, C3, C1q, kappa, lambda, fibrinogen, and C4d. Light microscopy features of biopsies were scored by Banff criteria on all biopsies.

### Study Endpoints

The primary outcome variable was reduced renal function (defined as a 1.5-fold increase in serum creatinine from baseline). Secondary endpoints were incidence of biopsy-confirmed chronic antibody-mediated rejection (CAMR) and recurrent GN.

### Statistical Analyses

Statistical analysis was performed using SPSS statistical software (version 21.0; SPSS, Chicago, Ill, United States). Results are reported as mean ± standard deviation when normally distributed or as median (interquartile range). Comparisons of continuous variables between 2 groups were assessed by using the unpaired *t* test or the Mann-Whitney *U* test, where appropriate. Pearson $\chi^2$ was used to test for differences among categorical variables. Univariate survival comparisons were made by using the log-rank test. Renal survival was analyzed by the Kaplan-Meier method, and this duration for each patient was computed from baseline evaluation to the last follow-up or the primary endpoint. Variables previously found to affect renal survival in the present study were included in the multivariate Cox proportional hazards model. Variables were selected by backward elimination using likelihood ratio tests. A $P$ value $< .05$ was considered statistically significant.

## RESULTS

Demographic characteristics of the study and control groups are shown in Table 1. The study and control groups were matched with regard to age, gender, and post-tx follow-up ($P = .407$, $P = .762$, and $P = .237$, respectively). There were no differences regarding initial serum creatinine levels (1.24 ± 0.30 vs 1.32 ± 0.37, respectively) between the study and control groups ($P = .441$). Only 1 patient in each group had a positive panel-reactive antibody test before tx ($P = .619$).

The patient and control groups were followed for a median of 53.5 (interquartile range 26.7 to 83.7) months. There were no differences in 12-month serum creatinine levels (1.25 ± 0.37 vs 1.27 ± 0.67, respectively) between the study and control groups, but there was a statistically significant difference at the end of follow-up (1.57 ± 1.1 vs 1.27 ± 0.47; $P = .286$ and $P = .003$, respectively). Patients who had positive auto-B FCXM had significantly reduced renal function compared with the control group ($P = .016$; Fig 1).

Three patients in the study group (3/35, 8.5%) and 5 patients in the control group (5/57, 8.7%) developed post-tx panel-reactive antibody during follow-up. Four patients developed biopsy-confirmed CAMR (11.4%, n = 4) in the study group and 1 patient (1.8%, n = 1) in the control group ($P = .047$). In subgroup analysis, 2 patient had biopsy-confirmed CAMR in the GN study group (2/11, 18%), and only 1 patient had CAMR (1/14, 7.1%) in the GN control group ($P = .026$).

The patients in the study group had a significantly higher incidence of recurrent GN after kidney tx (5/35, 14%) compared with the control group (1/57, 1.7%; $P < .001$). In subgroup analysis, 5 patients had biopsy-confirmed recurrent GN (5/11, 46%) in the GN study group, and only 1 patient (1/14, 7.1%) had recurrent GN in the GN control group ($P = .026$). Patient who had positive auto-B FCXM match had a significantly higher incidence of recurrent GN compared with the control group ($P = .038$; Fig 2). One patient (1/35, 2.8%) in the study group and 2 patients (2/57, 3.5%) in the control group developed biopsy-confirmed cell-mediated rejection ($P = .736$).

The incidence of new-onset diabetes after tx (NODAT) was significantly higher in the study group (7/35, 20%) compared with controls (n = 0, 0%; $P < .001$).

**Table 1. Demographic Characteristics of Study and Control Group**

| | Study Group (n = 35) | Control Group (n = 57) | *P* Value |
|---|---|---|---|
| Mean age (y) | 41 ± 12.6 | 37 ± 11.2 | .407 |
| Sex | | | |
| Female | 14 (40%) | 21 (36.8%) | .466 |
| Male | 21 (60%) | 36 (63.2%) | |
| Etiology of CKD (n) | | | |
| Glomerulonephritis | 11 (31.4%) | 14 (24.5%) | |
| Nonglomerular diseases | 12 (34.2%) | 12 (21.1%) | .157 |
| Others | 12 (34.2%) | 31 (54.4%) | |
| Post-tx follow-up (mo) | 57 ± 36 | 54 ± 30 | .237 |

Abbreviations: CKD, chronic kidney disease; tx, transplantation.

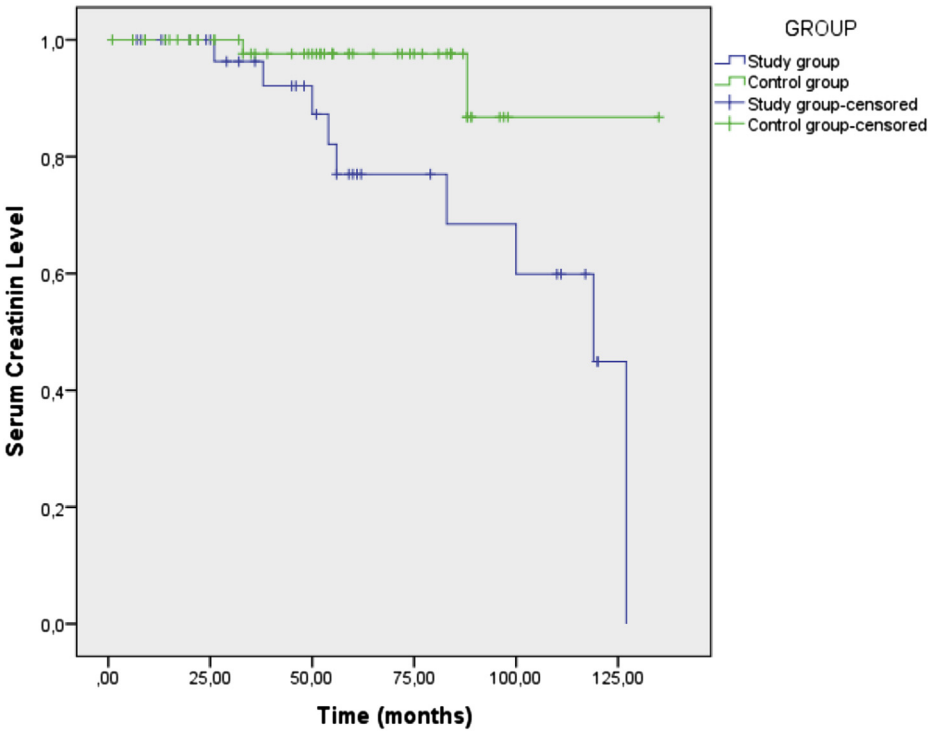



**Fig 1.** Effect of auto-B-cell flow cytometric auto-crossmatch on kidney function.

## DISCUSSION

The crossmatch test has been developed to identify anti-HLA antibodies in the recipient. Anti-HLA antibodies are also associated with graft survival and CAMR. However, many patients suffered from rejection and graft loss even in the absence of such antibodies. Some of the non-HLA antibodies that are previously described can affect kidney survival [5–7]. We think that some non-HLA antibodies

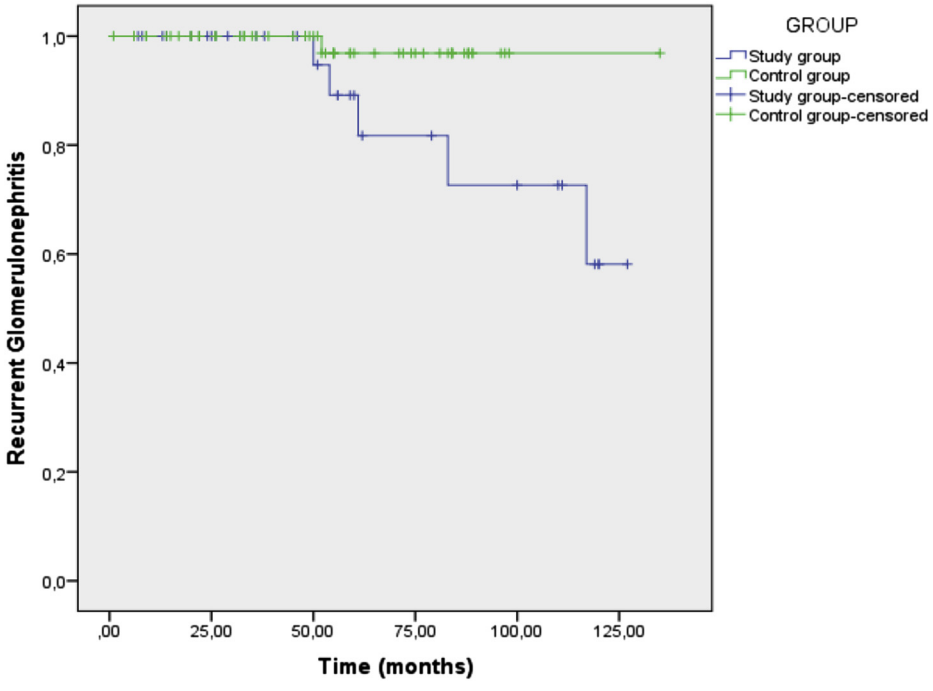



**Fig 2.** Effect of auto-B-cell flow cytometric auto-crossmatch on recurrent glomerulonephritis.

cause positive auto-FCXM results and may cause graft failure.

Autoantibodies can be directed either against kidney-specific autoantigens or against ubiquitous antigens in systemic autoimmune diseases with renal manifestations. Primary membranous nephropathy or Goodpasture disease develops because of some well-known autoantibodies [8–10]. However, some autoantibodies that cause some GN, such as focal segmental glomerulosclerosis and C3 glomerulopathy, have not been identified yet. Several undetectable autoantibodies may result in positive auto-FCXM test.

In the present study, we matched kidney tx recipients of low immunologic risk with a control group. Kidney transplant recipients with positive auto-FCXM test had significantly reduced renal function and a higher incidence of recurrent GN and CAMR compared with the control group. NODAT was significantly higher in the study group, which may be a result of high-dose immunosuppression.

The strengths of the current study include antibody testing, the single-center interpretation of tests, and robust matching of the study groups with controls. Limitations of the study are small sample size and short follow-up.

## CONCLUSIONS

The findings of this study suggest a potential role of autoantibody causing positive auto-FCXM test, increasing the risk of CAMR, recurrent GN, and NODAT. Further studies are required to clarify this issue.

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