Excellent Results of Immunocomplex Capture Fluorescence Analysis-I for Cross-Match Test in Renal Transplantation


ABSTRACT

A flow cytometry cross-match (FCXM) test is the gold standard for detection of human leukocyte antigen (HLA) antibodies in renal transplantation because of its high sensitivity. However, this technique can produce false-positive results when non-HLA antibodies or low-titer donor-specific antibodies (DSA) are detected. To determine the clinical relevance of the recently introduced novel cross-match test termed immunocomplex capture fluorescence analysis (ICFA), we retrospectively compared the results of ICFA and FCXM, including a single-antigen bead test for detection of DSA in renal transplant recipients. We found a correlation of 71.4% (235/329) between the results of ICFA-I and FCXM-T, whereas that between ICFA-II and FCXM-B was 41.1% (134/326). Ninety-four patients were ICFA-I negative and FCXM-T positive, and 188 were ICFA-II negative and FCXM-B positive, whereas 46.8% (44/94) and 61.7% (116/188) were found to be DSA-I and DSA-II negative, respectively, which classified them into the non-HLA antibody and low-titer DSA groups, respectively. The mean value of molecules of equivalent soluble fluorescence for DSA-I was 22,994 in the ICFA-I-positive group, which was significantly higher than 2117 in the negative group (P < .0001), whereas there was no significant difference for DSA-II between the ICFA-II-positive and ICFA-II-negative groups. Graft survival in the ICFA-I–negative group was significantly higher than that in the ICFA-I–positive group (P = .0058). Our results indicate that ICFA-I does not respond to non-HLA antibodies or low-titer DSA, which have influence on graft survival. Therefore, this novel hybrid test, which combines cross-match testing and HLA antibody detection functions, may be useful for clinical pretransplantation evaluation of renal transplantation patients.

COMPLEMENT-dependent cytotoxicity (CDC) cross-match (XM) testing has been routinely performed to detect the presence of human leukocyte antigen (HLA) antibodies in renal transplantation patients for more than 40 years since the landmark study by Patel and Terasaki presented in 1969 [1]. However, the CDCXM test is not sensitive enough to detect low levels of HLA antibodies, whose presence is associated with antibody-mediated rejection (AMR) [2]. Given that limitation, the more sensitive technique, termed flow cytometry XM (FCXM), has recently become the gold standard in many centers for detection of HLA antibodies in renal transplantation cases. On the other hand, due to its high sensitivity, this technique can produce false-positive results when non-HLA antibodies or low-titer donor-specific antibodies (DSA) are detected, which result from nonspecific binding of irrelevant antibodies to the cell surface [3,4]. More recently, a novel XM test termed immunocomplex capture fluorescence analysis (ICFA) was introduced. To determine the clinical relevance of its findings, we retrospectively compared the results of ICFA and
FCXM, including a single-antigen bead test (SAB) for detection of DSA in renal transplant recipients.

MATERIALS AND METHODS
ICFA Principles

ICFA was performed according to the protocol of the manufacturer (WAKFlow HLA antibody class I&II, Wakunaga Pharmaceutical Co., Ltd., Japan). The principle of ICFA is based on discrimination in selection of HLA immunocomplex from the antigen-antibody immunocomplex by coculturing donor lymphocytes and recipient serum samples with anti-HLA class I and II antibody Luminex beads. The cut-off value was calculated on the basis of the median fluorescence of each bead using an equation similar to that of the Luminex single antigen technology, a value >2.0 is used for determining positivity.

ICFA Protocol

With ICFA, patient serum (20 μL) is added to donor lymphocytes and incubated for 30 minutes at 37°C. During the mixture procedure, HLA and non-HLA antibodies in the recipient serum react with donor lymphocytes. After incubation with lysis buffer, 25 μL of the lysate is transferred to another 96-well plate containing HLA class I and II antibody Luminex beads, then incubated for 20 minutes at room temperature. After washing with buffer, the beads are incubated with anti-human immunoglobulin (Ig)G. In the present study, flow cytometric analysis was performed using a Luminex system.

Comparison of Results Between ICFA and FCXM

Three hundred twenty-nine ICFA tests were done between November 2009 and February 2012 using sera collected from 91 recipients at 4 transplantation centers (Hyogo Prefectural Nishinomiya Hospital, Hyogo College of Medicine, Kobe University Graduate School of Medicine, and Shimane University Faculty of Medicine). Tests of each sample were from 1 to 62 times. ICFA was used for preoperative and postoperative routine examinations, or evaluation of desensitization therapy. For evaluation of the accuracy of ICFA for detecting anti-donor antibodies, we compared the results between ICFA class I (ICFA-I) and T-cell FCXM (FCXM-T), and ICFA class II (ICFA-II) and B-cell FCXM (FCXM-B) testing. When mismatched cases were shown by 2 methods, we investigated the cause of the discrepancy using SAB. To evaluate the accuracy of DSA detection, the molecules of equivalent soluble fluorochrome (MESF) values obtained using SAB were compared with ICFA results.

Comparison of Graft Survival Between Pretransplantation ICFA-Positive and ICFA-Negative Recipients

From February 1999 to December 2012, 109 recipients underwent kidney transplantation at the 4 transplantation centers noted previously. We compared graft survival in a retrospective manner between pretransplantation ICFA-positive and ICFA-negative recipients.

RESULTS

Comparison of Results Between ICFA and FCXM

We found a correlation of 71.4% (235/329) between the results of ICFA-I and FCXM-T (both positive, 49; negative, 186) and of 41.1% (134/326) between ICFA-II and FCXM-B (both positive, 19; negative, 115), whereas 94 were ICFA-I negative and FCXM-T positive, and 188 were ICFA-II negative and FCXM-B positive (Table 1). In cases with results that differed between 2 methods, 46.8% (44/94) and 61.7% (116/188) were found to be DSA-I and DSA-II negative, respectively, and were classified into the non-HLA antibody and low-titer DSA groups, respectively (Table 2). The MESF value for DSA-I was 22,994 in the ICFA-I positive group, which was significantly higher than that in the ICFA-I negative group (Cr, 1.62 mg/dL; eGFR, 47 mL/min/1.73 m²) and those in the negative group (Cr, 1.26 mg/dL; eGFR, 60.4 mL/min/1.73 m²) (Table 3). There was no significant difference for DSA-II between the ICFA-II positive and ICFA-II negative groups (Fig 1).

Comparison of Graft Survival Between Pretransplantation ICFA-Positive and ICFA-Negative Recipients

Graft survival in the ICFA-I negative group was significantly higher than that in the ICFA-I positive group (P = .0058; Fig 2), whereas there was no significant difference for the results of ICFA-II. There were no significant differences between average serum creatinine (Cr) and estimated glomerular filtration ratio (eGFR) values of 30 days after transplantation in the positive group (Cr, 1.26 mg/dL; eGFR, 60.4 mL/min/1.73 m²) and those in the negative group (Cr, 1.62 mg/dL; eGFR, 47 mL/min/1.73 m²; P = .47 and .6, respectively).

DISCUSSION

Recently, for preoperative antibody examinations of renal transplantation cases, we generally evaluate the results of
FCXM by considering the results of DSA testing such as SAB. However, those tests are time consuming and have high costs. In contrast, ICFA is a new XM testing method used to selectively capture DSA in a coculture antigen-antibody immunocomplex composed of donor lymphocytes and recipient serum, with the use of anti-HLA antibody Luminex beads. This hybrid antibody measurement method combines the advantages of direct XM testing and DSA measurement by SAB. ICFA became available in Japan as a kit from Wakunaga, Pharmaceutical Co., Ltd. in July 2010. The cost of a single sample is quite low and results can be obtained within about 2 hours by use of a simple procedure. In this study, we found that ICFA did not react to non-HLA and low-titer HLA antibodies, and a significant positive correlation between the results of ICFA-I and MESF values was also noted. Furthermore, graft survival in the ICFA-I-negative group was significantly higher than that in the ICFA-I–positive group. Therefore, we consider ICFA-I to be superior XM testing and clinically effective to detect meaningful DSA levels.

Rituximab, a chimeric anti-human CD20 agent, is now widely used for desensitization of ABO blood group incompatible renal transplantation and treatment of AMR. The variable region of rituximab binds to CD20. CDC is mediated by activation of the complement cascade by the Fc portion of anti-CD20, which ultimately results in assembly of the membrane attack complex and cell lysis [5]. However, rituximab use has been associated with false-positive FCXM results [3]. On the other hand, ICFA-I is not influenced by rituximab, because T-cell lymphocytes do not express the CD-20 antigen. Therefore, ICFA-I is valuable for XM testing in patients treated with rituximab.

In the present study, we found that neither ICFA-I or ICFA-II reacts with non-HLA and low-titer HLA antibodies. However, there were no significant positive correlations between the results of ICFA-II and MESF values, as shown in Figure 1. It is possible that ICFA-II is not sufficiently captured by HLA class II, resulting in a false-negative result because there are not enough B cells expressing HLA class II in peripheral blood, namely, the amount of class II antigen is low. Another reason may be that positive ICFA-II cases are few, as shown in Figure 1 (4 in ICFA-II-positive cases, 79 negative). Therefore, it is necessary to consider increasing the donor lymphocytes and number of cases in relation to ICFA-II.

In conclusion, our results indicate that ICFA-I can detect clinically significant DSA only without a response to non-HLA and low-titer HLA antibodies, and that the results of ICFA-I are correlated to graft survival. We expect that ICFA, a novel hybrid test that combines XM testing and HLA antibody detection functions, will come into wide use.
use in the future for clinical pretransplantation evaluation of renal transplantation patients.

REFERENCES


