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HLA antibody screening strategy in patients awaiting kidney transplantation

Research Article

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Abstract: Background: The reactivity between donor's Human Leukocyte Antigen (HLA) and recipient's anti-HLA antibody in pretransplantation assessment is one of the critical factors to determining successful outcome of renal transplantation. Objective: The aim of present study was to compare different techniques of HLA antibody detection in patients waiting for a kidney transplant. Methods. Two techniques of HLA antibody screening were compared: the complement-dependent citotoxicity (CDC) test and enzyme-linked immunosorbent assay (ELISA). The study included 606 sera samples of 236 patients waiting for a first kidney transplantation. Results. Of 606 tested sera, 469 (77.39%) were negative by both methods. Of the 137 (22.6%) positive sera, 73 (12.04%) were positive only by ELISA method, 48 (7.92%) by both CDC and ELISA methods and 16 (2.64%) only by CDC method. There was a significant ($p<0.05$) correlation between optical densities obtained by ELISA and the PRA determined by cytotoxicity testing. Conclusion. Fast and precise characterisation of antibodies in patients before transplantation can be performed by both methods, CDC and ELISA, as complementary techniques. ELISA method is more sensitive and effective than CDC, enabling significant reduction of the CDC workload of the laboratory, but can be used only as adjunct to serum screening by citotoxic testing.

Keywords: *Antibodies • Human Leukocyte Antigens • ELISA • Kidney transplantation*

1. Introduction

Sensitization is defined as the presence of preformed alloantibodies (usually anti-HLA class I antibodies, but also anti-HLA class II or non HLA antibodies) in the serum of a prospective transplant recipient. The association between the presence of preformed cytotoxic antibodies to donor HLA antigens in kidney recipients sera and the development of graft rejection after transplantation has been well known for more than 30 years [1-3]. The clinical significance of anti-HLA antibodies has been documented since Dr. Terasaki's observation in 1969 that the presence of antibody correlated with rejection [4,5]. Today there are a wide variety of antibody screening methodologies. The results obtained with these various methodologies have played an important role in increasing transplant graft survival. The advances of methodologies for antibody screening were fueled by three main needs: improved sensitivity, improved specificity and the need for more automated, less subjective methods.

There are two main types of methodologies for detecting anti-HLA antibodies: antigen non-specific (i.e. cytotoxicity NIH, AHG, etc., and cell-based flow cytometric) and antigen specific (ELISA, bead-based flow cytometric, multiplex Luminex). The cell-based methodologies are not only antigen non-specific, but they are also time consuming and subjective. In the past, cytotoxic antibodies identification has been impaired by: (1) inability to distinguish between IgM and IgG antibodies; (2) correct identification of anti-HLA class II antibodies, especially in the presence of anti-HLA class I antibodies; (3) inability to detect the antibodies hidden by linkage disequilibrium. For example, in case of lymphocytotoxic tests, if all cells from a panel have HLA-B8 and HLA-A1 phenotypes, due to linkage disequilibrium, it is difficult to say that the patient has anti- HLA-B8 or anti-HLA-A1 antibodies or both antibodies types [6]. Additionally, there are several problems inherent in the CDC assay that compromise the accuracy of the test results. The effectiveness of rabbit serum, used as a source of com-

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plement and heterophile antibody, may vary greatly, that can affect the sensitivity. The CDC test does not differentiate HLA specific from other cytotoxic antibodies, and finally, because of extensive time and labor involved in maintaining a panel, testing sera and analyzing results, there is a high costbenefit ratio for tests of sera lacking HLA-specific antibodies [7]. The addition of antihuman globulin enhances the sensitivity of the assay by cross-linking the antibodies (AHG-CDC). In recent years, solid phase assays have been introduced as methods for HLA antibody screening which basically fall into two categories, namely ELISA-based methods and HLA antigen-coated beads used in either a Flow Cytometry system or a Luminex platform, are more sensitive than CDC for detecting both HLA class I and class II antibodies since it can detect binding of serum antibodies to specific antigens independently of complement activation and more, single antigen methods allow identification of an unique HLA specificity [8-10]. Also, with these new technologies, using additional anti-IgM/IgG antibodies, is possible to distinct between IgM and IgG anti-HLA antibodies [6,11,12]. A further improvement in antibody detection technique was reported by Yabu et al. They tested antibodies for their capacity to fix C1q complement and compared them to regular IgG antibody detection and found a higher specificity for the C1q technique in detecting antibodies associated with poor graft outcomes [13].

Among the various limitations, CDC remains the "gold" standard approach to characterize anti-lymphocyte antibodies. The aim of the present study was to evaluate and compare the abilities of two techniques (CDC and ELISA) to detect anti-HLA antibodies.

2. Methods

The study included 606 sera samples from 236 dialysed patients (154 males and 82 females, with an median age of 42.5 years) waiting for a first kidney transplant. An informed consent of the individuals participating in the study was obtained and all institutional ethics requirements were met. Every three months, all patients waiting for a kidney transplant send us a serum sample and we study it by CDC and/or ELISA methods [14-16]. The CDC assay was performed on a panel of T (or total) lymphocytes from 40 donors selected from a panel of HLA typed, peripheral blood lymphocytes, to be representative of HLA-A,-B and -C antigen frequencies in our population. Breafly, 1 µl of serum and 1 µl of lymphocyte suspension were incubated for 30 minutes at 22°C and then following addition of 5 µl of rabbit complement, for 1 hour. After second incubation, cells were stained with commercially available stain consisted of ethidium bromide and acridine orange and trays were examined by fluorescence microscopy to determine percent cell death. Approvment of HLA antibody specificity we accomplished by at least two same HLA antigens contained in the panel of lymphocytes. Sera showing either a defined HLA specificity or a positive reaction with at least 10% of the panel lymphocytes, we considered as positive. CDC positive sera were further investigated using dithiothreitol (DTT) to distinguish the presence of IgG or IgM antibodies [17]. The ELISA technique was performed by using commercial QuickScreen (QS12G) kits (GTI, Waukesha, WI) and OneLambda Antigen Tray Mixed kits (OneLambda Inc.,Canoga Park,CA) for detecting HLA class I-specific antibody. The test were performed according to the manufacturer's instructions:

1) Quick Screen,GTI: 50 µl of control or test serum, diluted with specimen diluent solution, was added to duplicate wells in a microtest plated and incubated for 40 minutes at 37°C. The plate was than washed with 300 µl of wash solution per well, and 50 µl of alkaline phosphatase–conjugated, affinity–purified goat antibody to human immunoglobulins (1:100 dilution) was added to each well. After incubation for 40 minutes at 37°C and three additional washes, 100 µl of p-nitrophenyl phosphate solution diluted 1:100 in the enzyme substrate buffer was added, and mixture was incubated in the dark at room temperature (18 to 22°C). The reaction was stopped after 30 minutes by the addition of 100µl of ELISA stop solution, and absorbance of each well at a wave-lenght of 405 nm was measured in an ELISA plate reader.

2) OneLambda Antigen Tray Mixed kits: 24 µl of test serum diluted 1:2 with specimen diluent solution was incubated with 28 HLA Class I and 22 Class II extracted antigens coating microplate and then alkaline phosphate conjugated anti-human IgG was added to develop the color changes in wells with specific antigen and antibody reaction.

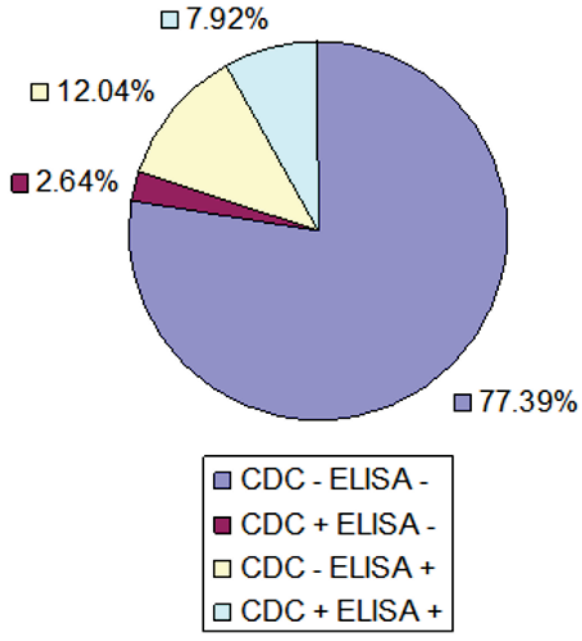
In this study we compared the results of CDC with ELISA class I antibody determinations. Satistical analysis was performed with the software Microsoft Office Excel 2002 for Windows. Pearson's *chi-squared test* (χ^2) was performed to evaluate the sensitivity of both techniques in detecting present lymphocytotoxic and/or HLA specific antibody. Correlation coefficient r was used to determine the correlation between the optical densities obtained in ELISA and the percent panel reactive antibody determined by cytotoxic testing.

3. Results

A total of pretransplantation 606 sera were evaluated for presence of HLA class I antibodies. 469/606 or (77.39%) sera were negative with both techniques, 48/606 (7.92%) serum samples were positive with both techniques, 89/606 (14.68%) were positive with at least one technique: 73 (12.04%) only with ELISA (data only for HLA Class I, while for HLA Class II data was not shown) and 16 (2.64%) only with CDC, respectively. In 16/16 of the CDC positive and ELISA negative sera, the capacity of DTT to negative the CDC positive result, indicated the presence of antibodies of IgM isotype. The screening results are shown in Figure 1.

Among CDC positive sera (64 or 10.56%), the peak PRA was between 5 and 20% in 23/64 (35.93%), between 21 and 60% in 27/64 (42.18%) and >61% in 14/64 (21.87%). The correlation between optical density mean values, obtained by duplicate testing of each sera by ELISA method, and percent panel reactive antibodies is presented in Figure 2.

Correlation coeficient was r = +0.357, signifficancy of correlation coefficient was t = 4.393, which in comparison to border value of 2.02, degree of freedom (V-2=48-2=46) and p<0.05, points to correlation between percent panel reactive antibodies and mean optical density values. This moderate correlation also means that the results obtained by ELISA method provide the information about the extent of sensibilisation.



CDC + ELISA Ø (16/16= IgM isotype)
χ^2= 17.56, p<0.05, border value=5.991

Figure 1. CDC and ELISA HLA Class I screening results.

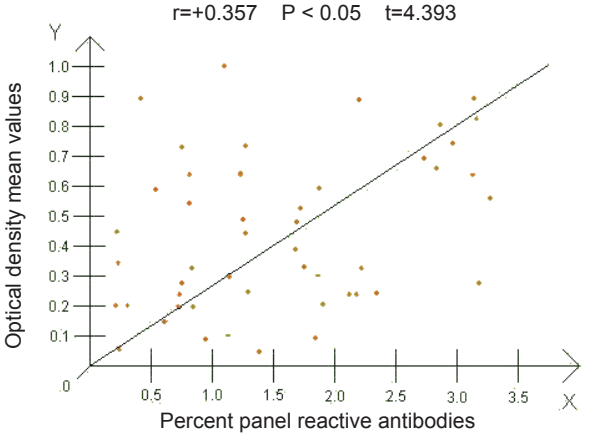


Figure 2. Correlation coefficient between percent panel reactive antibodies and mean optical density values.

4. Discussion

In kidney transplantation, the presence of preformed antibody against donor HLA, predispose hyperacute and acute post-transplant rejection and it is closely related with poor survival. The association between antibody response and acute immunological complications after transplantation has been under intense investigation in recent years [14,18,19]. The introduction of new technologies such as ELISA, flow cytometry and Luminex, has provided alternative methods to complement dependent cytotoxicity test for HLA specific antibody detection [20-22]. In this study, different methods used to detect HLA antibodies showed discrepant results. More “sensitive” technique was ELISA, which detected 19.96% class I positive sera, while CDC technique detected 10.56%. Additionaly, disadvantages of CDC are it’s inability to detect non-complement fixing antibodies and the fact that it detects auto-reactive antibodies that are not relevant to the transplant outcome. The ELISA method was able to detect a number of antibody presence significantly more frequently than the CDC method (19.96% vs 10.56%, χ^2= 17.56, border value is 5.991). The ELISA QuickScreen and OneLambda assays represents a valuable technique for routine screening of anti-HLA antibodies due to it’s higher sensitivity compared to the CDC technique. All CDC positive and ELISA negative sera (16/16) contained IgM antibodies. Therefore, ELISA assay could replace the CDC for regular transplant screening as well as for the testing selected patients whose first CDC test was negative and whose history does not reveal immunized events. The ELISA

method is rapid, simple, sensitive and specific, with no interference with autoantibody or non-HLA antibodies. In contrast, ELISA, screening does not obviate the need for percent PRA determinations, due to it's inability to detect IgM antibodies, in the cases when are not in usege additional anti-IgM/IgG antibodies. Therefore, we consider this complementary strategy, which includes useage of both CDC and ELISA techniques, simultaneously or alternatively, as appropriate and useful for HLA antibody screening in all pretransplant patients, since it provides the advantages of both of techniques, in conjunction. Our data are in agreement with other reports [18,23-25], that also confirmed the higher sensitivity and greater ability to detect antibodies by ELISA technique in comparison to CDC. This discrepancy of positive test results obtained only by ELISA 121 (19.96%), in comparisom to the positive results obtained only by CDC test 64 (10.56%), could be explained by either a greater sensitivity of the ELISA or to the presence of noncytotoxic antibodies which CDC method could not detect.

Our results showed moderate correlation (r = +0.357) with statistical significancy of r (t = 4.393), between the optical densities obtained by QuickScreen and OneLambda kits and percent panel reactive antibodies. Therefore, GTI QuickScreen and OneLambda Antigen Tray Mixed kits, although designed only to determine the presence or absence of HLA class I specific antibody, also provide an information of the extent of sensitization. This is another justification for complementar and adjunct usage of ELISA method to serum screening by cytotoxic testing. This complementary screening strategy also produce substantial saving of time and can provide the useful information that indicates the immunological status of patients.

5. Conclusion

Utilization of complementary HLA antibody screening strategy can result in a substantial cost saving, decrease the personnel time needed, enabling the detection of HLA specific antibodies.

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