International Journal of Clinical and Diagnostic Pathology

ISSN (P): 2617-7226
ISSN (E): 2617-7234
www.patholjournal.com
2020; 3(1): 01-05
Received: 01-11-2019
Accepted: 03-12-2019

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Comparison of cell based and solid phase assays in detecting pre transplant donor specific antibody and impact of its results on graft outcome

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DOI: https://doi.org/10.33545/pathol.2020.v3.i1a.144

Abstract
Background: The clinical significance of positive results on more sensitive platforms available for antibody screening has been a matter of contention. This study explores the relationship of test results of Complement Dependent Cytotoxicity (CDC) crossmatch, ELISA anti HLA antibody test and Donor Specific Antibody (DSA) assay using donor lysate on the Luminex platform and their impact on graft outcome.
Materials and Methods: Participants included patients who underwent renal transplant over four years, in whom pre transplant screening included an initial CDC cross match, followed by final CDC cross match, ELISA and DSA screening by the Luminex on the final pre transplant serum. Relevant clinical data and results of supportive laboratory investigations were taken from HLA laboratory and computerised records.
Results: 126 recipients were included in the study. Pre transplant CDC positivity correlated with overall rejection episodes (P=0.03) and the historical crossmatch Ig M/G positivity with biopsy proven rejections (P=0.029). There was no significant correlation between pre-transplant DSA and ELISA results and rejection episodes. A comparison between DSA, ELISA and CDC showed a low association (Kappa value for DSA and CDC: -0.42; ELISA and CDC: - 0.63; DSA and ELISA: + 0.159).
Conclusion: CDC continues to have relevance in pre transplant screening. In spite of their technical advantages and superior sensitivity, the ELISA pooled antigen assay and the Luminex crossmatch do not appear to show any significant clinical advantage when used with conventional CDC.

Keywords: Donor specific antibody (DSA) assay, complement dependent cytotoxicity (CDC) crossmatch, anti HLA antibody test, Luminex assay, renal transplant

Introduction

Despite the effective immunosuppressive regimens, Chronic allograft dysfunction poses a great challenge to renal transplantation [1]. Post transplant denovo Donor Specific Antibodies (DSA) against foreign graft HLA are strongly associated with antibody mediated graft failure [2].

Pre transplant anti HLA antibodies are generally known to be deleterious for a renal graft [3]. They are responsible for hyper acute rejections, some acute (antibody mediated) rejections and are believed to play an indirect role in chronic rejections. The Complement Dependent Cytotoxicity (CDC) test has been traditionally used for screening for pre-transplant anti HLA antibodies and its outcome has been a gold standard for years to determine whether a patient may safely proceed for transplant [4]. However, many newer, more sensitive platforms are now available for antibody screening, including the ELISA and Luminex solid phase However, the clinical significance of positive results on these more sensitive platforms when the CDC is negative, has been a matter of contention [5]. This study attempts to explore the relationship of test results of CDC crossmatch, ELISA anti HLA antibody test and DSA assay using donor lysate on the Luminex platform and their impact on graft outcome.

Materials and Methods

Institutional Ethics committee approval was obtained before commencing the study. Participants included patients who underwent renal transplant in our centre over four years, in whom pre transplant screening included an initial CDC cross match, followed by final

CDC cross match, ELISA and Donor Specific Antibody (DSA) screening by the Luminex crossmatch (Luminex donor specific antibody test using donor lysate) on the final pre transplant serum. Relevant clinical data for the recipients were collected retrospectively and prospectively from computerised case summaries available from the clinical workstation and transplantation records. Results of supportive laboratory investigations were taken from HLA laboratory and computerised records.

CDC cross match was done on both autologous and donor (B and T unseparated) lymphocytes using extended incubation timings, serial dilutions to detect prozone, and dithiotreitol (DTT) treatment to define isotype. Test wells that showed 5% or more dead cells were reported as positive. Current and historical CDC results with the same donor were analysed. The ELISA test was performed on a mixed antigen platform (LATM; One Lambda, USA) and analyzed in terms of positivity or negativity (based on manufacturer defined calculations involving negative control well optical density (OD)), as well as OD values for class I and II antibodies.

Luminex DSA (Lifecodes, USA) was performed according to the manufacturer's protocol and test results were analyzed in terms of positivity or negativity for class I. Median Fluorescent Intensity (MFI) exceeding 1000 was categorized as positive.

Post transplant laboratory values of Serum creatinine, 24hrs urine protein, glomerular filtration rate (GFR) values at 7th day, 14th day, 1 month, 3 months, 6 months, 12 months, 18 months, 2 years, 3 years, 4 years and 5 years, details of graft loss were documented. The maximum follow up period was 60 months. All patients with suspected rejection episodes had renal allograft biopsy performed on them. The biopsies were interpreted and graded using the Banff 07' classification.

Post-transplant complications in terms of delayed graft function, acute rejections, number of rejection episodes, renal allograft histopathology details during each episode (cellular or vascular rejection), and any histological evidence of chronic rejection were included in analysis as end points.

Results

126 recipients were included in the study.

Results of historic and final CDC cross match tests

Historic CDC crossmatch was positive in 18 cases, out of which 10 were positive for IgM alone and 8 were positive for IgM with coexistent IgG (IgM/G) and none were positive for IgG alone. The final CDC crossmatch showed 11 positive cases and all 11 were positive for IgM alone and negative for IgG alone or IgM/G.

Results of ELISA test

Of the 126 recipients, 22(17.46%) had ELISA positivity. Out of these 22, 19 (15.04%) had positivity for Class I antibody, 9 (7.14%) had positivity for Class II antibody, 6 (4.76%) had positivity for both Class I and II antibody.

Results of pretransplant DSA assay

Of the 126 recipients, 6 (4.8%) had anti-Class I DSA antibodies, 21(16.7%) had anti-Class II DSA antibodies and 5 (4%) had both class I and class II DSA antibodies.

Tables 1 and 2 show the relationship of pre-transplant screening with various outcomes. Pre transplant CDC positivity correlated with overall rejection episodes (P=0.03) and the historical crossmatch Ig M/G positivity with biopsy proven rejections (P=0.029). There was no significant correlation between pre-transplant DSA results and rejection episodes, though there was an apparent association between DSA positivity with 14th day eGFR (P=0.05). OD ELISA class I showed a significant correlation with 6th month eGFR (P=0.01).

Odds ratio was quite significant for CDC (4.108) in predicting rejection episodes and not for DSA (0.972). When DSA was combined with CDC in analysis, the odds ratio was not significantly improved (4.108 to 4.117).

A comparison between DSA, ELISA and CDC (tables 3-5) showed a low association (Kappa value for DSA and CDC: - 0.42; ELISA and CDC: - 0.63; DSA and ELISA: + 0.159).

Discussion

Detection of pre-transplant anti-HLA antibodies in a recipient is aimed at protecting the recipient from the risk of antibody mediated rejection. However it can also label him or her as "sensitized," reducing transplantation options. The decision of denying transplantation in these patients is neither ethical nor economical if allosensitization proves clinically irrelevant or manageable.

Overall, current recommendations suggests that pre-transplant DSA detected exclusively in other assays with a negative CDC, should not be a contraindication but rather a risk factor [3, 6] and that solid phase antibody screening assay results should always be interpreted in conjunction with crossmatch results and other details to determine whether they are immunologically significant or not [3]. Furthermore factors like strength of the antibody, subclass of the immunoglobulin and its complement fixing capacity play a role in deciding which antibodies are clinically significant [7, 8].

In our study, both initial and final pre-transplant CDC crossmatches (P=0.03) predicted rejection episodes. Historic CDC crossmatch (for either IgM/G) positivity predicted biopsy proven rejection episodes (P =0.029). Patients with evidence of pre transplant IgG with or without IgM in the final pre-transplant crossmatch were not cleared for transplant and hence would not have been included for analysis. However our findings show that historical positivity for IgG even in the presence of an immediate pre transplant CDC that is negative for IgG poses a risk for rejection. While a few studies report that recipients who are current CDC crossmatch negative, but historic CDC positive have no added risk of graft rejection [9, 10], others have reported an impact of historical positivity on outcomes causing it to be included as a criterion for upgrading immunological risk [11]

Conventional CDC cross match is often criticized for its technical constraint of requiring viable lymphocytes from the donor, varied techniques leading to lack of consensus in results, and rather subjective interpretation, as well as for its lack of specificity and sensitivity. It may detect clinically irrelevant antibodies to other antigens on the lymphocyte surface, in addition to HLA. As regards sensitivity, it will detect only antibodies that mediate complement dependent cell lysis. Complement fixing antibodies are considered to be more of a threat than non- complement fixing antibodies

[12-14]. Yet antibodies without complement fixing activity are known to be capable of graft destruction through complement independent mechanisms, and have been identified in elutes of rejected kidneys. Moreover, because of its low sensitivity, CDC assay detects only high levels of antibody.

In spite of these limitations, CDC has consistently demonstrated correlation with transplant outcomes, based on which there has arisen a consensus of pre-transplant CDC positivity being a contraindication to transplantation [15, 16]. At the same time, positive CDC crossmatch due to autoreactive cytotoxic antibodies, which are mostly IgM are not considered a contraindication to transplantation [17-20]. Our study, on the other hand suggests that even alloreactive IgM antibodies, when detected on the CDC crossmatch immediately prior to transplant, may be associated with rejection.

In contrast to CDC, solid phase assays do not require vital cells, detect both complement and non-complement fixing antibodies, are technically more standardized, and offer objective semiquantitative interpretations. The Luminex crossmatch (DSA testing based on donor lysate derived HLA coated beads) for detecting pre-transplant DSA, uses antigen extracted from donor lysates, thereby performing a real donor-recipient crossmatch on the Luminex platform. This provides the advantage of detecting existence of DSA using natural human antigen, without additional donor typing, besides making it cheaper than comparable tests using precoated beads. However, HLA C, DQ and DP antigens are not effectively captured in its current format, and consequently antibodies to antigens belonging to these loci are not detected. False positive results have also been described due to antibodies against the beads [21]. Further, there is no consensus for defining cut-off MFI values leading to a wide variation between all laboratories performing this assay.

Pre-transplant DSA detected by ELISA and Luminex in CDC crossmatch negative cases in our study did not independently predict rejection episodes, graft loss and 1 year post transplant 24 hour urine protein during the study period. However there was some correlation with eGFR noted transiently in the post transplant period and this marker has been shown to have some predictive value for graft loss in the long term.

Both ELISA and Luminex platforms have much higher sensitivity for antibody detection compared to CDC, Luminex more than ELISA, and they also detect non complement fixing antibodies. Whether this exquisite sensitivity and the detection of non-complement fixing antibodies contribute to the lack of real correlation with clinical outcomes evident in our own as well as some other studies is to be considered. The likelihood of routine potent pre-transplant immunosuppressive induction protocols in our study, protecting against the impact of low level antibodies detected on these platforms alone, cannot be excluded. In our centre, candidates who are even 5% positive in the CDC crossmatch are considered sensitized and this very low cutoff may have further reduced the clinical impact of additional solid phase testing.

Both the solid phase platforms used in our study, by design identify only Ig G isotype anti- HLA antibodies [22], and this limitation may have further contributed to the lack of statisitical correlation with rejection in our study, given the apparent clinical impact of IgM antibodies noticed on the final pre transplant CDC.

Other studies have reported a correlation of Luminex and ELISA with post transplant outcomes [23-25]. These included mostly patients who received grafts from cadaveric donors where immunological and other factors that determine outcome may differ from our cohort who received transplants primarily from live and related donors. Moreover, as far as Luminex goes, many of these studies were done using the single antigen bead assay, a different format which uses precoated beads and can detect C, DQ and DP antibodies.

Table 1: Relationship of variables with eGFR at various time intervals

Variables	7 th day eGFR Mean/PC [P Value]	14th day eGFR Mean/PC [P Value]	1st Month eGFR Mean/PC [P Value]	3rd Month eGFR Mean/PC [P Value]	6th Month eGFR Mean/PC [P Value]	1year eGFR Mean/PC [P Value]
DSA positive	65.4 [0.45]	68.4[0.05]	71[0.19]	68.2[0.94]	66.7[0.98]	65.4[0.49]
DSA negative	61.0	61.1	65.5	67.9	66.6	68.8
ELISA positive	66.9 [0.35]	60[0.47]	64.6[0.56]	67.1[0.8]	64.4[0.49]	65.6[0.6]
ELISA negative	61.9	63.5	67.4	68.2	67.1	68.5
O.D ELISA Class I	-0.108[0.22]	-0.160[0.07]	-0.137[0.12]	-0.154[0.08]	-0.225[0.01]	- 0.002[0.98]
O.D ELISA Class II	0.017[0.84]	-0.011[0.9]	-0.043[0.63]	-0.045	-0.048[0.59]	-0.038[0.68]
Pre-transplant CDC positive	58.2 [0.48]	60.1 [0.59]	67.1[0.96]	61.9[0.23]	64.8[0.71]	55.5[0.09]
Pre-transplant CDC negative	63.3	63.3	66.9	68.6	66.8	69.1
Historical CDC Ig M positive	61.6 [0.86]	61.1[0.75]	66.3[0.92]	68.8[0.88]	68[0.8]	62.3[0.21]
Historical CDC IgM negative	63	63.1	67	67.9	66.5	65.2

Abbreviations: DSA,Donor specific Antibody; O.D- Optical Density; CDC, Complement Dependent Cytotoxicity; eGFR – Estimated Glomerular Filtration Rate; PC – Pearson Correlation

Table 2: Relationship between variables with the outcome

Variables	Rejection Episodes No./Mean [P Value]	Biopsy proven rejections No./Mean [P Value]	Acute vascular rejection No./Mean [P Value]	Graft loss No./ Mean [P Value]	1yr GFR <45ml / 24hrs No./ Mean [P Value]	1yr 24hr Ur. protein > 300 mg No./ Mean [P Value]	1yr GFR<45ml or 1 yr 24 hr Ur. protein >500mg No./Mean [P Value]	1 yr 24 hr Ur. protein > 500mg Mean/PC [P Value]
Total Positive cases	**32**	**12**	**7**	**7**	**10**	**22**	**16**	
DSA Class I positive	3 (0.46)	2(0.28)	1(0.48)	0(1)	2(0.21)	1(0.83)	2(0.55)	173.8(0.45)
DSA Class II positive	7(0.75)	3(0.85)	3(0.2)	1(0.6)	3(0.47)	3(0.82)	4(0.78)	200.9(0.47)
ELISA Class I positive	2(0.15)	0(0.21)	0(0.59)	0(1)	2(0.65)	2(0.51)	2(1)	149.8(0.15)
ELISA Class II positive	1(0.28)	0(0.59)	0(1)	0(1)	1(0.58)	1(1)	1(1)	174.7(0.48)
Pre-transplant CDC positive	6(0.03)	2((0.28)	1(0.48)	1(0.3)	1(0.58)	3(0.37)	1(1)	208.8(0.74)
Historical CDC Ig M positive	3(0.69)	3(0.02)	0(1)	0(0.43)	1(0.58)	3(0.37)	2(0.32)	232.9(0.58)

Abbreviations: DSA, Donor specific Antibody; CDC, Complement Dependent Cytotoxicity; eGFR – Estimated Glomerular Filtration Rate; PC, Pearson Correlation

Table 3: Comparison of Final pre-transplant CDC and DSA test results in the recipients

DSA	CDC		Total
	Positive	Negative	
Positive	2	30	32
Negative	9	85	94
Total	11	115	126

Abbreviations: DSA, Donor specific Antibody; CDC, Complement Dependent Cytotoxicity

Table 4: Comparison of Final pre-transplant CDC and ELISA test results in the recipients

Elisa	Cdc		Total
	Positive	Negative	
Positive	1	21	22
Negative	10	94	104
Total	11	115	126

Abbreviations: ELISA – Enzyme Linked Immunosorbent Assay; CDC, Complement Dependent Cytotoxicity

Table 5: Comparison of DSA and ELISA test results in the recipients

Dsa	Elisa		Total
	Positive	Negative	
Positive	9	23	32
Negative	13	81	94
Total	22	104	126

Abbreviations: DSA, Donor specific Antibody; ELISA – Enzyme Linked Immunosorbent Assay

Conclusion

Our results indicate that CDC continues to have relevance in pre transplant screening. Moreover IgM antibodies detected by CDC immediately prior to transplant show an association with rejection that merits further study since these antibodies are generally described as harmless and not a contraindication for transplant. In spite of their technical advantages and superior sensitivity, the ELISA pooled antigen assay and the Luminex crossmatch do not appear to show any significant clinical advantage when used with conventional CDC.

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