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RESEARCH ARTICLE

Luminex Crossmatch for Pre-Transplant Workup of Renal Transplants - 30 Months Experience from Indian Subcontinent

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Abstract

Background: Prior to 2013, most centers in India performed only Complement Dependent Cytotoxicity crossmatch (CDCXM) for detection of donor specific antibodies.

Aim: This study was undertaken to evaluate the usefulness of Luminex based crossmatch (LXM) for pretransplant detection of HLA class I and II donor specific IgG antibodies in patients with end stage renal disease.

Methods: Luminex Crossmatch was performed on samples from 654 patients, which included 582 first time recipients and 72 retransplants. CDC crossmatch was done for all 654 recipients. Panel Reactive Antibody (PRA) screen was performed for 352 (53.8%) recipients. Low resolution HLA typing was done by reverse Sequence Specific Oligonucleotide probes on Luminex platform.

Results: Twenty-five recipients (3.8%) had a positive CDCXM. LXM was positive in 277 samples (39.2%): HLA -class I donor specific antibodies (DSA) were detected in 40 (5.6%), class II DSA in 155 (21.9%) and both classes in 82 (11.5%) samples. High background was present in 70 (9.9%) samples - the results of which were confirmed by retesting a fresh sample or by correlation with PRA screen.

Conclusions: LXM in combination with PRA screen is useful for detection of clinically relevant HLA IgG donor specific antibodies for pretransplant workup and is a huge improvement on CDCXM.

Keywords

Luminex crossmatch, Complement dependent cytotoxicity crossmatch, Panel reactive antibody screen, Pretransplant, End stage renal disease

Introduction

Luminex X-Map technology has revolutionized anti-HLA antibody detection globally enabling detection of low level antibodies, which were not detected previously by the conventional Complement Dependent Cytotoxicity crossmatch (CDCXM). Luminex - X-Map technology is being used globally for anti-HLA antibody detection using four kinds of kits: pooled, phenotype, single antigen bead assay and luminex crossmatch (LXM) using 5.6 microns size polystyrene beads [1]. LXM has gained wide acceptance in the Indian subcontinent because of its ability to detect low levels of sensitization, ease of use, ability to characterize as HLA - class I or II reactivity and its price. Preformed donor specific antibodies have been implicated in hyperacute rejection, acute rejection and chronic renal allograft nephropathy [2]. An important guideline of the Rome Consensus Conference is that both solid phase and cell-based methods should be used for pre-transplant detection of donor specific antibodies [3]. Before availability of Luminex bead-based crossmatch assay in mid-2013, most centers in India performed only complement dependent cytotoxicity crossmatch (CDCXM) prior to transplant. Flow cytometry crossmatch (FCXM) is not used widely in India due to a lack of uniformity in reporting and variation in cut offs used to assign positivity. HLA typing is very often not done for renal transplants involving biologically unrelated donors in many private hospitals in India.

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Luminex based single antigen bead (SAB) assay first became available in 2015, but its price in commercial centers was three to four times the cost of LXM with additional cost of DNA typing. In combination with PRA screen LXM has proved a cost effective technique. In India, where > 95% of renal transplants are from a living donor, and health insurance does not cover the entire cost of diagnosis and treatment so the test price is of critical significance [4].

In practice a 'real' crossmatch is nearly always possible with minimal delay, because even in deceased donor transplants, most organs are shared within the same city. The pre-transplant immunogenetic work up in many centers, especially in the private sector, until recently included only CDCXM with often no HLA typing for biologically unrelated transplants, but many nephrologists have now adopted LXM for detection of sensitization and for monitoring immunosuppression. LXM (trade name LIFECODES Donor Specific Antibodies), a propriety product of Immucor, Stanford CT, USA, is a solid phase immunoassay which is available in India. The test does not require viable cells as it utilizes a lysate made of donor cells isolated by density gradient centrifugation, which can be stored at ≤ -20 degree Celsius for future testing. It is easy to interpret and costs less than one quarter of SAB assay. One limitation of LIFECODES Donor Specific Antibodies (LIFE CODES DSA) is that it detects only anti HLA -A, HLA -B, and anti HLA DR IgG antibodies [5]. Anti HLA class I and or class II antibodies detected are due to reactivity against the corresponding class of HLA antigens unlike FCXM and CDCXM where positive B cell crossmatch can occur due to either low levels of HLA class I antibodies or HLA class II antibodies [6]. Luminex bead-based antibody assays have almost completely replaced the technically demanding ELISA based methods.

Most centers in Europe and United States perform a combination of tests of which single antigen bead (SAB) testing is most important for antibody specification and is often preceded by a pooled bead assay for screening. The SAB assay, despite being relatively expensive, can facilitate a 'virtual crossmatch'. Additionally, DNA typing for HLA-A, B, C, DR, DQ and DP alleles of the donor is required for interpretation of the clinical relevance of SAB results. If no donor specific antibodies are detected, a final crossmatch can be omitted, which reduces cold ischemia time with substantial benefit to the patient. However, SAB assays can be falsely positive in unsensitized individuals which has been attributed to denatured HLA molecules on the surface of the beads and naturally occurring antibodies by various workers [7,8].

This study was undertaken to evaluate the usefulness of LXM as a strategy for detecting sensitization in patients with ESRD. The results were correlated with sensitization history, with other results of additional antibody detection tests and DNA typing if available. LIFE CODES DSA was validated against CDCXM, Flow crossmatch and PRA specification prior to its implementation, and the laboratory has been participating in the College of American Pathologists external proficiency testing scheme (CAP EQAS) since 2013. The EQAS results of LXM were matched with that of FCXM because no EQAS was available which tests for LXM. The results were matched in 90% of the samples over a two-year period [9]. LXM is essentially a qualitative test and some centers simply report it as positive or negative, while other centers prefer to report in terms of MFI (mean fluorescence intensity). Billen, et al. reported that LXM class II DSA does not contribute towards graft failure and although class I DSA is not associated with increase in acute rejection episodes, it may result in lowered long-term graft survival [10].

Materials and Methods

This study was undertaken in the HLA department of National Reference laboratory of Dr. Lal Path Labs Ltd., New Delhi during the period June 2013 to December 2015. This laboratory processes samples from many transplant centers in India, Nepal and Bangladesh. As all were outsourced samples from commercial hospitals, it was not easy to obtain fresh samples or payment for additional tests including repeats. No funds were allocated for this study and data was derived from tests performed for clinical care.

A total of 748 samples were received from 697 recipients and 720 prospective donors for LXM. Subsequently 43 recipients were excluded from the study because of inadequate history or/and insufficient testing for correlation. The study therefore included 705 samples from 654 recipients and 697 donors. Ten samples were submitted for inter-lab comparison of LXM (data not shown). Forty-seven recipients tested by LIFE CODES DSA had DSA with high MFI and were retested after desensitization; ten of them against multiple donors. Immunosuppression protocols differed in various centers depending on the state of sensitization, nephrologists personal preference and patients' paying capacity. It included one or more of: Plasmaphereses, Rituximab, Mycophenolate Mofetil, Antithymocyte Globulin, Tacrolimus and Bortezomib. Desensitization was done with ATG for MFI < 3000 with addition of plasmapheresis if MFI was higher. All Luminex based assays were performed on Luminex 100 (Luminex Corporation, USA).

DNA typing

Low resolution HLA- A, B, DR typing was performed by reverse-sequence specific oligonucleotide probes on Luminex. Target DNA is PCR-amplified using biotinylated group-specific primers. Less than 5% of the samples were typed for HLA-C and HLA-DQB1. A single PCR reaction is used for each HLA locus. The biotinylated PCR product bound to the microsphere is labelled with

streptavidin conjugated with R-phycoerythrin (SAPE). A flow analyzer identifies the fluorescent intensity SAPE on each microsphere. Software is used to assign positive or negative reactions based on the strength of the fluorescent signal. Assignment of the HLA typing is based on positive and negative probe reactions compared with published HLA gene sequences. A negative control was included in each batch of DNA that was tested.

CDC B and T cell crossmatch: Was performed for 550/654 recipients on B and T cells isolated using nylon wool, from peripheral blood mononuclear cells by Extended NIH method [11]. Dithiothretiol treated serum was used for detection of alloreactive IgG antibodies and untreated serum for detection of IgM auto antibodies. Serum was tested in doubling dilutions up to a titer of 16 to allow for detection of any prozone effect. Of seven patients with historical positive crossmatch five had a current negative CDCXM. Samples from 104 recipients were tested in-house in the referring hospital.

PRA screen (Pooled Bead assay): For detection of HLA class I and II IgG antibodies was performed using LMX Life codes screen Deluxe kits (Immucor, USA) which utilizes seven and five beads coated with multiple HLA antigens for detection of HLA class I and II IgG antibodies respectively, as per manufacturer's instructions. Although it is essentially a screening test, we validated patient sera reactivity against the beads, and graded the reactions as weak, intermediate and strong based on the number of beads reacting and their mean fluorescence intensity (MFI). The grading was validated for fifty samples against phenotype assay kits from the same vendor, showed acceptable correlation and proved cost-effective. Pooled bead assay was performed for 172/277 DSA positive (62.1%) and 180/377 DSA negative (47.7%) recipients.

Phenotype bead assay

LIFECODES Quik-ID Class I and LIFECODES Quik-ID (Immucor USA) were used for identification of specificities of anti HLA antibodies in 40 samples. Test and analysis was performed as per procedure mentioned in product insert.

Single Antigen Bead Assay was done on 12 samples retrospectively on samples that had already been tested by LXM, pooled and phenotype assay as a part of validation process of the test and it became available only towards the time of data compilation.

Luminex crossmatch: Was performed using kits for detection of HLA class I and II IgG DSA as described by Huh, et al. with a modification that lysate control was not used for each sample after validation of the test [12]. Interpretation was done by Quick type software initially and subsequently by the Match IT! Antibody software. The steps of the test were briefly as mentioned below:

- Donor lysate is prepared from peripheral blood mononuclear cells separated by density gradient centrifugation. 8 µl of donor lysate and 5 µl of beads are incubated in the dark for 30 minutes at ambient room temperature [RT] (20-240 Celsius). This serves as a target for serum anti-HLA antibodies.
- Donor HLA coated beads are incubated with serum diluted in specimen diluent allowing an antigen-antibody reaction.
- Following another wash, the diluted anti-Human IgG Phycoerythrin (PE) conjugate is added.
- After half an hour incubation at RT, a final wash is given and the plate is then placed in the Luminex for acquisition of data and analysis.

Internal Quality Control was in the form of known negative and positive controls in all runs, with lysate controls in some runs. Auto LXM was done for five samples that gave unexpected class II positive DSA against donors who were HLA identical for DRB1 locus at low resolution.

Interpretation

A positive LXM result was considered **true positive** if one or more of the following were present:

a) Presence of anti HLA antibodies was supported by another method such as CDC, PRA screen, PRA quantitative, flow crossmatch or SAB.

b) Concordance on serial testing or inter-lab comparison

c) Definite history of sensitization including pregnancy, transfusion or a previous transplant

d) Serial declining values showing response to treatment or consistence of values if refractory.

A LXM was considered **negative** if no antibodies were detected, or even if LXM was positive with either of the following:

a) Negative PRA screen.

b) Reactivity against HLA identical antigens in first degree relatives which could be explained as auto positivity after testing.

LXM is essentially a qualitative test with the results generating a Mean Fluorescence Intensity (MFI) for HLA - Class I and II IgG DSA. Reporting was done as positive or negative and further graded as weak (MFI 500-1000)/intermediate (MFI 1001-2000)/strong (MFI > 2000). Final assignment of a test result as positive was made only if the MFI was > 500 and if two or more of the beads gave a positive result.

Statistical Analysis Epi-info: 7 software was used for statistical analysis of accrued data. P values were calculated for DSA in first time versus retransplants, male versus female recipients for both first time and retransplants.

Table 1: First time recipients and retransplants patient - Donor characteristics, Sensitization status and high background samples

Parameter	1st Transplant n = 582	Retransplant n = 72
Recipient age: Range (mean) years	13-72 (42.9)	22-67 (42.9)
Donor age: Range (mean) years	23-70 (42.3)	27-75 (47)
Female recipients **n = 213** (32.6%)	202 (33.1)	11 (21.1)
DSA negative	97	02
DSA positive **n = 114** (53.5%)	105 (52.1%)	09 (81.8%)
Class I	23	0
Class II	51	9
Both	31	0
Male recipients **n = 441** (67.4%)	380	61
DSA negative	259	19
DSA Positive **n = 163** (37%)	121 (31.8%)	42 (68.9%)
Class I	12	5
Class II	75	20
Both	34	17
Female donors **n = 371**	348 (59.8)	26 (36.1)
Donor profile **n = 705**	612	93
Unrelated	234 (38.2)	50 (53.8)
Parents	116 (18.9)	6 (6.4)
Siblings	80 (13.1)	5 (5.4)
Spousal	127 (20.8)	11 (11.8)
Off-spring	05 (0.8)	06 (6.5)
Non-first-degree relatives	45 (7.4)	15 (16.1)
Deceased Donor	05 (0.8)	0
High Background (n = 70)	65	5

Table 2: DSA and positive CDCXM in First time recipients and Retransplant

	1st Transplant	Retransplant	p value
DSA positive female recipients	105/202 (52.1%)	09/11 (81.8%)	0.01
DSA Male recipients	121/380 (31.8%)	42/61 (68.9%)	0.0000
Current positive CDC crossmatch (Positive/Total)	21/582 (3.6%) N = 582	4/72 (5.5%)	0.626
DSA positive (all males and females)	226/612 (38.8%)	51/93 (70.83%)	0.000

Results

Patient characteristics

The age of patients ranged from 13-72 years (mean 43.5); mean donor age was 42.8 years (range 23-75) years. Male recipients (67.4%) outnumbered females, but the percentage of female donors was higher (53.7%) which shows common gender distribution for renal transplants in India Table 1 shows the patient donor profile. There were 284 (40.2%) unrelated donors in the study including 21 (3.2%) paired exchange transplants. There were only five deceased donor transplants in this study - a reflection of low deceased donor transplant rates in India.

CDC crossmatch: Was positive in the current sample of 21 first time recipients (3.6%) and in four (5.5%) requiring retransplant. LXM was positive in all patients with current positive CDC crossmatch, but was negative in three of the seven patients with historical positive CDC crossmatch. Absence of any HLA IgG in these samples was further substantiated in the current negative samples by performing PRA screen. Of the 25 current CDCXM positive samples tested for DSA, 24 had MFI > 8000 for HLA - Class I IgG DSA. CDCXM positivity was not significantly more frequent in retransplants (Table 2).

HLA IgG antibodies: Were detected by PRA screen in 187 (51.7%) of the 352 samples (Table 3). No HLA IgG antibodies were detected by PRA screen on 50/70 (70%) of the samples which gave a high background (HBG) with LXM and thus the antibody test was useful in assigning a negative DSA result to these samples. PRA

Table 3: Details of Immunogenetic work up and results for 654 Recipients

Test	1st time recipients	Retransplants
Current positive CDC crossmatch (Positive/Total)	21/582 (3.6%)	4/72 (5.5%)
PRA screen - Number (%)	320 (54.9)	32 (44.4)
Negative	152 (47.5)	13 (40.6)
Positive (n = 187)	168 (52.5)	19 (59.4)
Class I positive	46 (27.4)	05 (26.3)
Class II positive	40 (23.8)	04 (21.1)
Both positive	82 (48.8)	10 (52.6)
PRA specification	35 (70.8)	10
Single antigen bead test	7	5
DSA negative	412	21
DSA positive (Luminex Crossmatch)	226	51
Class I	35	5
Class II	126	29
Class I and II	65	17
False positive (Luminex Crossmatch)	54	
Class I	3	
Class II	42	None
Class I and II	9	

Note: The false positive results which are actually negative are shown separately, but are included in negative results.

Table 4: Details of 12 patients tested for DSA following desensitization.

S. No	Age	Sex	1st/ ReTx	No. of Donors	DSA I	DSA II	Response of DSA to Desensitization	Outcome
1	39	F	2nd	5	152-1309	551-10763	Class I & II reduced	Transplanted
2	52	M	2nd	10	1230-10096	1600-14200	Refractory	Not Transplanted
3	43	F	1st	7	5421-11863	Negative	Refractory	Not Transplanted
4	45	M	3rd	3	Negative	342-2600	Reduced	Transplanted fine 4yr later
5	35	M	3rd	4	Negative-	12000-19000	Refractory	Not Transplanted
6	54	M	1st	1	Negative	1100-2100	Reduced	Acute Rejection, Serum Creatinine 1.0 mg/dl at 1 year
7	52	F	1st	1	5680	8470	AMR	Died; DSA done retrospectively
8	39	M	1st	1	Negative	1292-3400	Reduced	Transplanted fine at present
9	62	M	1st	1	869	763	Acute rejection	Recovered, doing fine
10	31	F	1st	1	900	1100	Allograft dysfunction	Recovered, Biopsy negative
11	40	M	1st	1	Negative	450-4371	Declined	Transplanted, doing well
12	28	F	1st	1	1845-5382	Negative	Reduced	Transplanted Acute cellular rejection

Legend: AMR: Antibody mediated rejection; F: Female; M: Male; Tx: Transplant; ReTx: Retransplant; No: Number. Please note where two DSA values are mentioned, the values correspond to the range of MFI for different donors. Where there is a single donor it corresponds the value before and post immunosuppression.

screen result was considered as reference for deciding whether the LXM result was false positive because it includes adequate number of specificities and in our two years' participation (at the time of compiling the data) there was never a deviation from CAP EQAS results for PRA screen.

Donor specific antibodies: Were detected in 277 (39.2%) samples out of 705 that were tested. As expected a higher percentage of retransplants had positive DSA. The results of LXM including false positive results are depicted in Table 3. False positive results were obtained in 54 samples (7.7%) of which 42 (77.8%) were only HLA- class II DSA. The false positive samples although shown separately in Table 2 is included in negative results. Results of LXM were consistent in all 47 recipient samples (7.2%) that were tested more than once in that the same class of HLA antibody was

positive. Analysis of results of ten samples submitted for inter - lab comparison was concordant except for one HLA- class II result. Of the nine sensitized recipients that were transplanted following desensitization, five developed allograft dysfunction, of which four had biopsy proven rejection. Another patient (serial 7) who died was positive for HLA - Class I and II DSA on retrospective testing by LXM (Table 4).

Five samples that were auto LXM positive against their HLA identical first degree relative tested negative on PRA screen. Peak MFI of HLA - class I and II DSA was 21606 and 26654 respectively (data not shown). DSA as expected were significantly more frequent in retransplants as compared to first time recipients when overall and gender-wise data for distribution was analysed (Table 2).

High background

High background for negative controls was observed in 70 samples (9.9%) and made it difficult to interpret the results. It persisted on repeat testing on the same sample in forty samples. Repeat LXM testing was performed on fresh sample for 20 recipients; and the results were concordant with that of pooled bead assay, all of which were negative. An additional wash was also useful to lower the background without affecting control values on ten samples. In a commercial lab with high clientele pressure and low turnaround time (72 hrs), rather than delaying the results by asking for new sample, we carried out a PRA screen for correlation of results. PRA screen was negative for 50 samples with high background (negative control MFI > 500).

Additional Luminex assays included PRA specification and SAB on a total of 52 samples (7.9%) as described in a previous study. B and T Cell Flow crossmatch was performed for ten samples.

The SAB test and phenotype assay gave concordant results. PRA specification correlated with the class of DSA detected on LXM and with the HLA type of donor in addition to other anti-HLA antibodies.

Statistical analysis

The presence of DSA was statistically significant in retransplants as compared to first time recipients ($p < 0.001$) for both male and female recipients (Table 2). CDCXM positivity in the two groups was not significant ($p = 0.626$, Table 2). Although DSA were more prevalent in female recipients for both first time and retransplants, it was not statistically significant ($p = 0.6$) in the latter (Table 5).

Luminex crossmatch in sensitized recipients

Twenty-five CDCXM positive recipients, all with persistent DSA > 3000 were not considered for transplant because nephrologists did not want to take the risk of performing HLA incompatible transplant. The number of samples with only HLA class I, only class II and both MFI in excess of 10000 were 11, 13 and 7 respectively. Twenty-seven recipients with class I or/and class II DSA having MFI > 3000 were desensitized. In 22 patients the DSA as detected by LXM MFI was refractory and hence could not be transplanted in spite of a negative CDC crossmatch. Table 4 shows the antibody workup of 12 sensitized recipients.

Follow up

Information was obtained telephonically from all centers which had outsourced immunogenetic workup to this referral laboratory. As a part of service agreement, the centers approached this lab for query pertaining to any rejections that the recipients experienced. Four hundred and seven recipients were transplanted of which 169 had post-transplant suspected allograft dysfunction during six months-two years follow up [13]. Further evaluation of the recipients showed AMR (n = 30), acute cellular rejection (n = 23) and dual morphology (n = 2). The remaining recipients had non-immunological causes for allograft dysfunction.

Discussion

Characterization of a patient's HLA antibody profile can be extremely complicated, requiring sometimes a combination of tests on multiple samples. Both false positive and negative results can occur with any of the bead-based assays making it imperative to interpret results in conjunction with clinical history. In this study HLA-DSA were detected in 39.2% of patients, which is higher than that reported in a recent retrospective study from India where 25.4% had DSA when tested by LXM [14]. This may be because more sensitized patients are being transplanted and samples from these patients are received into tertiary referral center like ours.

Fifty-four samples (7.7%) gave a false positive result, most of which were HLA-class II DSA (Table 3). A higher cut off for unsensitized individuals can reduce the number of false positives. Negative controls gave a high background in 70 samples (9.9%) of which fifty

Table 5: P values for both genders for first and retransplants

	1st Transplant		p value
	Males	Females	
Luminex crossmatch (DSA)	121 (31.8%) n = 380	105 (52.1%) n = 202	0.00002
Retransplant			
Luminex crossmatch (DSA)	42 (68.9%) n = 61	09 (81.8%) n = 11	0.6

samples tested negative for PRA screen, enabling us to categorize DSA as negative. LXM is at best a semi-quantitative test but MFI values for DSA has made it more meaningful, helped for prediction of compatibility, assessing sensitization status and evaluating response to desensitization. Advantages of LXM over CDC include its higher sensitivity, no requirement for viable samples, and the ability to store lysate for future testing.

Prevalence of HLA-class II IgG DSA was much higher than HLA-class I IgG DSA but, the prevalence of both classes of HLA IgG antibodies was almost similar in PRA screen (Table 3). Two previous studies from India also reported a higher prevalence of class II IgG DSA [13,14]. Gulliuame, et al. reported that LXM was negative in samples positive for SAB class I with MFI up to 4100 and class II up to 1300 [5]. The authors however have not mentioned anything regarding outcome in these recipients.

Only two DSA negative recipients developed acute rejection which may have been due to acute cellular rejection or non-HLA antibodies. Further data was not available as histopathological features were equivocal and facilities for testing for non HLA antibodies are not available. The percentage of true positive LXM results in the population was similar to those of other workers that have tested using the SAB assay (Table 6). Flow cytometry crossmatch has yet to become popular in India, while LXM has been widely accepted as interpretation is easier. The role of flow crossmatch for primary renal transplants is controversial. BCFXM interpretation can be difficult due to non-specific fluorescence and lack of specificity [15,16].

There are a limited number of studies on LXM, most of which have also carried out additional antibody testing on CDCXM negative samples using SAB as gold standard. Billen, et al. observed the specificity of LXM for detection of HLA-class I and HLA-DRB1 donor specific antibodies to be 100 and 97% respectively when compared with FCXM and SAB assay. Eleven samples which were positive for HLA class I and ten samples that were positive for HLA class II DSA by LXM were negative by SAB assay which was attributed to higher density of HLA molecules on beads used in the LXM assay [17]. A study to compare LXM with SAB assay concluded that SAB was able to detect IgG antibodies in higher dilutions as compared to LXM in 76 and 97% of samples respectively [18]. Huh, et al. studied 55 patients with PRA > 20%, and found a higher incidence of acute rejection in LXM positive patients, all of whom developed acute cellular rejection [12]. Riethmüller, et al. concluded that Pretransplant class I antibodies detected by LXM but not class II are predictive of AMR and the sensitivity and specificity was 75 and 90% respectively at MFI of 900 [19]. One major limitation of this study is that the patients were from multiple centres which had outsourced the samples to our lab and hence complete follow up data is not available.

The difference in percentage positivity of samples in various studies could be due to the patient characteristics including ethnicity and difference in MFI cut off used for assigning a positive result. Table 6 compares the findings of seven available studies on LXM including present work.

All previous studies on LXM to date have suggested a doubtful role of LXM class II positive results in acute rejection and graft survival. Class II positive DSA were detected in 75% of retransplants and over 30% in first transplants. It is not possible for to comment on the impact of these antibodies on graft survival at this juncture as follow up data is limited.

The presence of anti-HLA antibodies in non-transfused males has been described previously but

Table 6: Salient features of contemporary studies on luminex crossmatch

Authors	No. of samples (% positive)	Class I positive	Class II positive	Both positive	Sample nature	Conclusions
Billen, et al. [10]	165 (19.4)	16	15	1		LXM more sensitive than FCXM
Huh, et al. [12]	55 (32.7)	6	5	7	PRA I/II > 20%	Sensitivity of LXM class I 62.5%, class II 100%
Mishra, et al. (present study) [8,13]	705 (39.3)	40	155	82	Pretransplant	LXM useful with PRA for detection of sensitization
Gulliuame, et al. [5]	106 (77.8)	26	28	30		LXM has low sensitivity for anti A and B
Caro-Oleas, et al. [18]	61 - all	-	-	-	Known sera & lysates	SAB more sensitive than LXM
Riethmuller, et al. [19]	155 (8.4)	7	6	-	PRA +	SAB I and LXM I are useful for AMR prediction
Vimal, et al. [14]	126 (25.4%)	6	21	5	Pretransplant	CDCXM neg DSA positivity no effect on short term outcome

Legend: CDCXM: Complement dependent cytotoxicity crossmatch; FCXM: Flow cytometry crossmatch; LXM: Luminex crossmatch; No: Number; +: Positive; PRA: Panel reactive antibodies; SAB: Single antigen bead test; DSA: Donor specific antibodies; &: and.

usually these are directed against HLA antigens with low frequencies and hence probably are not significant clinically [7]. This study showed that Luminex crossmatch in combination with PRA screen was successful and cost-effective strategy for detecting sensitization and also for monitoring immunosuppression, in patients with ESRD awaiting transplantation. With the limited follow up data available we conclude that < 5% of patients with negative LXM experienced acute rejection. This study suggests that a negative LXM test may be more meaningful than a positive test which must be correlated with additional history or testing. Detection of false positives limits the use of immunosuppression with its attendant risks. Finally, it is recommended that more multicenter studies be done on larger numbers of samples in combination with other antibody detection tests to determine its value in pretransplant work up, particularly where cost is a constraint.

Conclusion Luminex Crossmatch (DSA) which became widely available in India in mid-2013, has proved to be a huge improvement on CDCXM for detection of donor specific anti HLA IgG antibodies. It has been used for pre-transplant work up, evaluation/monitoring of extent of sensitization and is easy to interpret, cost effective in spite of its limitations most of which can be overcome if combined with a pooled bead assay.

Authors Contribution

Mahendra N Mishra has written the manuscript and analysed the data along with Dr. Vandana Lal. Dr. David Turner has helped by extensive proof reading the manuscript and has edited the contents. Dr. Puja Dudeja has performed statistical analysis of the data.

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