Доклади на Българската академия на науките
Comptes rendus de l'Académie bulgare des Sciences

Tome 78, No 6, 2025

MEDICINE

Immunology

LUMINEX CROSSMATCH IN KIDNEY TRANSPLANTATION – SINGLE CENTRE EXPERIENCE

Petya Yankova[1,2]✉, Anastasiya Mihaylova[1], Elisaveta Naumova[1]

Received on September 19, 2023
Presented by B. Petrunov, Member of BAS, on October 31, 2023

Abstract

Screening for preformed antibodies and prospective crossmatches are an essential component of pre-transplant testing. In this study, we evaluated the application of a Luminex solid-phase crossmatch (LumXm) as a routine test in clinical transplant practice. The test was performed with LIFECODES Donor Specific Antibody (DSA) kit (Immucor, USA) and verified using sera with known HLA specificities. For kit specificity and sensitivity testing we applied recipients' sera with defined HLA antibody specificities by Luminex Single Antigen bead (SAB) assays and cells from the potential donor. The clinical application was assessed comparing LumXm and flow cytometry crossmatch (FCXM) results in 44 real donor/recipient pairs. Concordant results were observed among FCXM, DSAs and LumXm in most donor/recipient combinations. The discrepancies in some cases were related to false positive HLA class II and false negative class I LumXm DSAs. In summary, the humoral alloimmune responses in transplantation should be interpreted carefully using a combination of assays, as well as the medical history of the recipient.

Key words: Luminex crossmatch, donor-specific antibodies, flow cytometry crossmatch, kidney transplantation, solid-phase assay

Introduction. Since the first demonstration that the hyperacute allograft rejection could be caused by preformed donor-specific antibodies (DSAs) by Patel and Terasaki in 1969 [1], the cell-based complement-dependent cytotoxicity

https://doi.org/10.7546/CRABS.2025.06.14

crossmatch (CDC-XM) has become an essential component of the pre-transplant testing in organ transplantation. Subsequently, more sensitive methods were introduced to improve the detection of donor-specific reactivity. The development of a CDC-XM assay using anti-human globulin as a secondary antibody in 1972, led to increasing sensitivity and reduction in false-negative rates of standard CDC-XM [2]. In 1983, a next generation of XM analysis was introduced – automated and objective semi-quantitative Flow Cytometric Crossmatch (FCXM) [3]. However, false positive results are common for this very sensitive cell-based XM assay. To overcome the limitations of the aforementioned assays, solid-phase tests applying purified HLA molecules were created for detection of anti-HLA reactivity [4, 5]. These more sensitive, specific and reproducible assays were quickly established into routine practice, replacing cell-based methods. Considering these advantages, solid-phase methods for cross matching were introduced, like Luminex crossmatch (LumXm), which uses donor-isolated HLA coated onto microbeads as targets for DSA binding. Methodological aspects and clinical significance of LumXm have been investigated by different authors, with some results being contradictory [6–12].

Based on our many years of experience with bead technology [13] and FCXM [14] in transplant immune-diagnostics, we considered that it is necessary to also evaluate the application of Luminex crossmatch as a routine test in the clinical transplant practice. To achieve our purpose we compared the results of donor-specific reactivity obtained by using Luminex Single Antigen bead (SAB) assays, LumXm, and FCXM.

Material and methods. ***Flow cytometric crossmatch*** was performed as previously described [14]. ***Antibody specificity*** was determined by Luminex SAB assays (LIFECODES LSA Class I & II; Immucor, USA). Samples fluorescence signals were measured by a Luminex 100 system. Data analysis was done by MATHCHIT v.13 software.

For ***Luminex crossmatch*** a LIFECODES DSA kit (Immucor, USA) was used following the manufacturer's instructions. This kit includes two groups of beads conjugated with specific for HLA class I or class II monoclonal antibodies intended to capture donor HLA molecules. Donor lymphocyte (approximately 2.2×10^6 cells per well) are lysed to release HLAs from the cell surface. For each well 8 µL of lysate (supernatant) were mixed with 5 µL of beads and incubated with the HLA coated beads. Bound DSAs are detected with anti-human IgG PE. The quality control system includes: a lysate control reagent to assure that HLA have been captured; a dried lymphocyte control (DLC) to be tested with a positive control, containing DLC specific antibodies and with a negative control serum; three control beads for monitoring the background. The samples were collected and analyzed on a Luminex 100 instrument with MATHCHIT v.13 software. A sample is classified "positive" or "negative" for DSA based on software calculated adjusted mean fluorescence intensity (MFI) values.

To verify the method, we used highly positive sera from an external alloantibody proficiency testing (Eurotransplant) with known HLA specificities, negative controls from HLA antibody detection kits, and donor cell lysates with HLA specificities matching these of the antibodies. The kit specificity and sensitivity were assessed by testing recipient sera with defined HLA antibody specificities by SAB assay and cells from the potential donor (Table 1). Finally, to evaluate the clinical application of this test we compared LumXm and FCXM results. Different donor/recipient combinations ($n = 44$) were chosen and tested with LumXm based on actual FCXM results consistent or discordant with expected (virtual) XMs (Table 2). In case of negative DSAs in routine screening but a positive prospective FCXM the alloantibodies were tested again in the XM serum sample.

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LumXm testing with sera with known HLA antibody specificities

No	**Donor's HLA**	**Recipient's HLA alloantibodies**	**LumXm DSA XM**	
			HLA class I	**HLA class II**
1	A*03,*24; B*39,*40; DRB1*04,*11; DQB*03.	Negative	Negative Score 1	Weakly positive, Score 2 (False positive?)
2	A***02**,***30**; B*13,***44**, DRB1*07,*16; DQB*02,*05.	A1,**2**,3,11,24,26,29,**30**,31, 33,34,36,43, 66,68,80; B**44**,45,57,58,76,82	Strongly positive Score 3	Weakly positive, Score 2 (False positive?)
3	A*02,*30; B*13,*44, DRB1***07**,*1**6**; DQB*02,***05**.	DR1,3,**7**,8,9,10,12,13,14,15, **16**;51; DQ**5**,6, DQA, DP	Negative Score 1	Weakly positive, Score 2 (MFI=1360)
4	A*24,*32; B*18,*35, DRB1*11,***16**; DQB***03**,*05.	DR103,3,4,7,8,9,**11,**12,13, 14,15,**16**; DR52,**53**; DQ2,6,**8(3)**, **9(3)**; DQA, DPB	Negative Score 1	Strongly positive Score 3 (MFI=5160)

Legend: Mismatched donor antigens and DSA are in bold

Results. The specificity and sensitivity testing showed somewhat unexpected and discordant results (Table 1). LumXm was positive in higher levels DSAs, with stronger reactions if DSAs are directed against more mismatched HLA. Surprisingly, we observed a very weakly positive HLA class II Luminex DSA reactivity in HLA class II negative sera.

Comparing FCXM, DSA, and LumXm data, results between the three tests were concordant for most donor/recipient combinations – in case of positive FCXM due to presence of DSAs, LumXm was also positive (data not shown). Also, Luminex HLA class II DSA test (patients 2 and 4) was negative in case of positive B-cell and auto-FCXM but lack of anti-donor reactivity. However, LumXm assigned unexpected positive results for HLA class II in four cases: patient 1 with negative

Table 2

Comparison of LumXM and FCXM results

Groups No	Patients No	LumXm		FCXM		Alloantibodies and other data
		HLA class I	HLA class II	T cells	B cells	
1	1	Neg	**Pos**	Neg	Neg	AlloAbs (-) (pregnancy-2; transfusions-2)
2A	2	Neg	Neg	Neg	**Pos**	HLA-class II Abs (+) but DSA(-); autoXM: T(-)/B(+)
	3	Neg	**Pos**	Neg	**Pos**	HLA-class II Abs positive, DSA Pos; autoXM: T(-)/B(+)
2B	4	Neg	Neg	Neg	**Pos**	HLA-class I Abs(+); DSA (-); autoXM: T(-)/B(+); Inflammation
2C	5	Neg	Neg	Neg	**Pos**	Abs(-); No data on sensitizations; autoXM: T(-)/B(-)
3	6	**Neg**	Neg	Pos	Neg	Low titers HLA-A2 DSA;MFI=2123; HLA-class II Abs (-)
	7	**Neg**	**Pos**	Pos	Neg	Low titers DSA – HLA-B56 MFI=1137; HLA-class II Abs (-)
4	8	**Neg**	**Pos**	Pos	Pos	Allele specific DSA-B35 MFI=1423; non-DSA HLA class II Abs; Previous transplant/transfusions

Legend: Abs – antibodies; MFI – mean fluorescence intensity; Neg – negative, Pos – positive; Discrepant results are in bold

both alloantibodies and FCXM; patient 3 – with positive B-cell and auto-FCXM but without DSAs detected; patients 7 and 8 – with non-DSA HLA class II antibodies. In an attempt to explain the results we performed a Lum auto-crossmatch in the patient with T(-)/B(+) auto-FCXM. Surprisingly, the auto-LumXm was classified as positive for HLA class II reactivity. For case 5 with inexplicable positive B-FCXM, Luminex DSA test demonstrated negative result corresponding to the patient's allosensibilization status. In patient 8, instead of the expected HLA class I positive result corresponding to the DSA profile from the SAB tests and FCXM, the LumXm determined the results as negative for class I DSA. In patients 6 and 7, the positive T-cell FCXM, explained by us to the presence of low-titer DSAs, was not confirmed by the LumXm test.

Discussion. The crossmatch strategy should define the immunological risk by distinguishing graft-damaging antibodies. The LumXm assay uses native antigens released from actual donor lymphocytes, Luminex solid-phase technology, and does not require viable cells, making it highly specific and sensitive in detecting antidonor reactivity. Data for the sensitivity of LumXm range from 54% to 96% for HLA class I and between 65% and 93% for HLA class II [7, 8]. The results for specificity appeared to be more consistent – 100% for HLA Class I and 97% for HLA Class II [7, 8]. Several authors note that the test detects HLA-DR

antibodies, but detection of HLA-DQ and DP antibody specificities is questionable or absent [7, 8, 10]. Moreover, LumXm has a low sensitivity for HLA-A and -B antibodies [8] and failed to detect anti-Cw reactivity [8, 10]. Furthermore, the comparison of FCXM, SAB and LumXm assays for detection of donor-specific reactivity showed no correlation of LumXm with FCXM and SAB assays, especially for the detection of class II DSA [15] or low agreement (53.8%) of the results between the three tests [16].

As for our preliminary data LumXm results are comparable to those of alloantibodies and FCXM at higher antibody titers. In subjects with T(-)/B(+) FCXM and T(-)/B(-) auto-FCXM but negative DSAs this test supports the absence of donor-specific reactivity. However, a disadvantage is the false positive HLA class II DSA reactions estimated by us and frequently found [10, 17] with the LumXm technology. To exclude non-specific positivity due to non-HLA antibodies Guillaume et al. [8] recommend auto-LumXm, which we also performed in our study. In addition, our finding of negative LumXm in individuals with positive low-titer HLA class I DSAs and T-FCXM is consistent with the data of Guillaume et al. [8]. Therefore, we consider it necessary that own criteria should be developed to determine the results of this test.

In summary, the discrepancies we observed between LumXm, SAB and FCXM in some cases support the opinion that humoral alloimmune responses in transplantation should be interpreted carefully using a combination of assays, as well as the medical history of the recipient. Despite the limitations described, LumXm may provide useful additional information for assessing compatibility in living kidney transplant donors and in haemopoietic stem cell transplantation, as well as for post-transplant monitoring especially in subjects on therapy affecting the results of the cell-based methods.

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[1]*Clinic of Clinical Immunology with Stem Cell Bank,*
"Alexandrovska" University Hospital, 1 St. Georgi Sofiiski St, 1431 Sofia, Bulgaria
e-mail: petya_yankova@yahoo.com, mihaylova_ap@abv.bg, naumovaej@gmail.com

[2]*Medical University – Sofia, 15 Akad. Ivan Evstratiev Geshov Blvd, 1431 Sofia, Bulgaria*