Review Article

Cross-matches in Transplantation: Each is Complementary to Other

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Abstract

Immune system, which is an obligatory component of body's defense mechanism, is the main barrier for allogenic transplantation. Perfect selection of donor on the basis of human leucocyte antigen (HLA) compatibility and cross-matching are the key components of successful transplantation. Cross-match eliminates the chance of hyper-acute rejection and also ensures the long term graft survival. In 1960s, T-cell complement-dependent cytotoxicity cross-match was firstintroduced; since then it is mandatory component of transplantation work-up process. With the advances of time different newer cross-match technics are evolved for determining the likelihood of donor-specific antibody-mediated responses, including flow cytometric cross-match and virtual cross-match using luminous microbead assay. Flow cytometric cross-match and luminous microbead assay are more sensitive and specific than the previous one, however all are complimentary to each other for final selection of donor recipient couple. This article builds an understanding of modern day cross-match interpretation using a complicated case-based approach.

Key word: *cross match; kidney transplantation.*

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Introduction

Renal transplantation is the best form of renal replacement therapy (RRT) for patients with end-stage renal disease (ESRD). It has an unequivocal superiority over dialysis.[1]

An organ other than from an identical twin when transplanted is rejected. This rejection is due to the interplay between immune system and non-self-antigen present on the donor's tissue. Immune system is so developed that it can sustain against millions of microbes in the environment. The key element of this defence mechanism is to recognise the microorganisms as non-self and destroy them. This defence mechanism which is otherwise vital for living is the main barrier to allogenic transplantation. Immune system comprises of two components- innate and adaptive immunities (Figure 1). Both are active in transplant rejection, innate acts non-specifically whereas adaptive immunity is activated specifically only when exposed to a non-self-antigen.[2]

Major Histocompatibility Complexes (MHC) is the key component of adaptive immune system, these are highly polymorphic cell surface polypeptides, which are encoded by cluster of genes presented on the short arm of chromosome six, in human it is called human leukocyte antigen (HLA). MHC Class I presents on virtually all nucleated cells, whereas Class II molecules are normally restricted to the antigen presenting cells. The primary role of MHC is to present the non-self-antigen to immune system. External antigens are incorporated with MHC class II and presented to CD4 T cells, whereas internal antigens like viral antigens, tumour antigens are presented with MHC class I to CD8 cells specific for that foreign antigens and thereby provoke the immune response and destroy them. (Figure 2)

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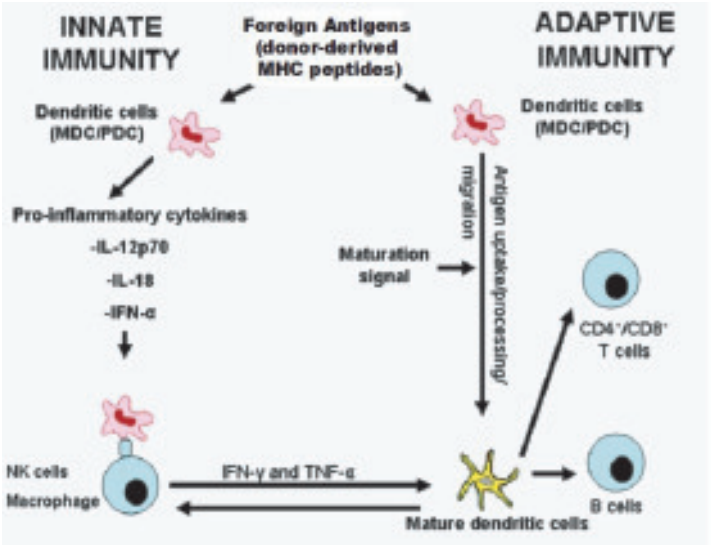
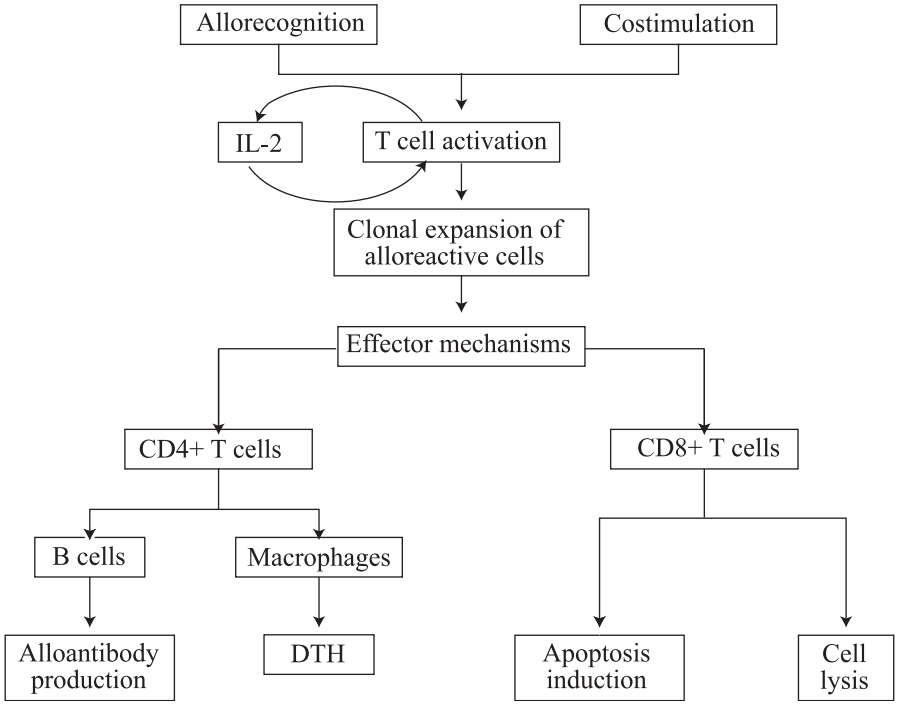


Figure-1: *Innate and adaptive immune response to foreign antigens.*[3]
[Adapted from Hung Do N et al. The Evolution of HLA-Matching in Kidney Transplantation, 2013]



Once T cell activation occurs, a chain of intracellular events is triggered under the influence of a variety of growth and differentiation factors, of which the best characterized is IL-2 these events lead to clonal expansion of antigen-specific T cells. Such cells are differentiated into CD8+ cytotoxic T cells (which directly induce donor cell death) and CD4+ helper T cells (which help B cells produce alloantibodies and help macrophages induce delayed type hypersensitivity responses). All of these mechanisms are active in graft rejection Schematic representation of intracellular signaling in graft rejection.

Figure-2: *Schematic representation of intracellular signaling in graft rejection [Adapted from Up To Date 21.2]*

There are three different class I (HLA-A, -B, -C) and class II (HLA-DQ, -DR, -DP) antigens. Antigenic barrier to transplantation is dependent on these antigens, especially HLA -A, B, and DR. Antigens encoded from these regions are the determinant for transplant rejection. For better graft survival, donor and recipient should be matched for these antigens. Two from each loci that is all six antigens should be matched for the best outcome.(4) Matching for DR locus has greater impact than others.(4) Allograft rejection can be categorised as hyper-acute rejection, acute rejection and chronic rejection, now termed as chronic allograft nephropathy. By adequate MHC matching and using effective immunosuppressive protocol the risk of acute rejection can be overcome which indirectly reduce the risk of chronic allograft nephropathy.[5,6] Hyper-acute rejection occurs due to preformed donor specific antibodies (DSA). Immediately after reperfusion these antibodies bind to the HLA antigens of graft vasculature and by activating the classical compliment cascade causes endothelial necrosis, platelet aggregation and local coagulation which ultimately ends up with rejection. These DSA are usually developed due to previous transplantation, blood transfusion and pregnancy.[7] Nowadays hyper-acute rejection is rare due to routine practice of pre-transplant crossmatching.

Clinical Scenario

History

A 30-year-old male patient with Chronic Kidney Disease 5 (CKD5) had been on haemodialysis (HD) for the last 5 years, secondary to lupus nephritis.

There was no history of blood transfusion or previous transplantation.

Transplant Status

The patient received the offer of a kidney from a deceased donor.

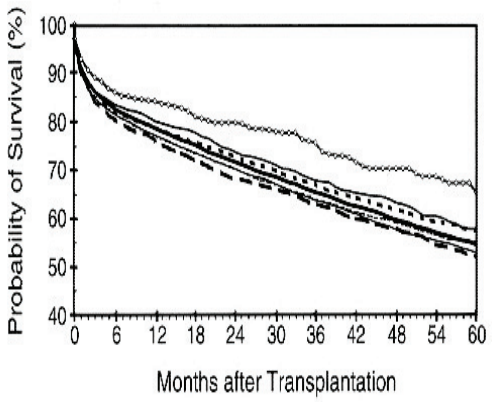
Laboratory results at admission for transplantation

His laboratory results when he was admitted to the unit for preparation for transplantation were as follows:

- 100 mismatch.
- Complement dependent cytotoxicity (CDC) crossmatch reported positive for B and T cell, but flow cytometry crossmatch (FCXM) was reported negative for both B and T.
- Luminex-SAB did not identify any Donor Specific Antibodies (DSA).

Discussion of the case

The cause of the patient's ESRD was SLE. There are conflicting data on transplantation outcome in SLE patients. Some studies showed SLE adversely affects the transplant outcome, (8-10) whilst others did not support this.[11-16] The largest retrospective study usingUnited StatesRenalData System (USRD) and United Network for Organ Sharing(UNOS) databases showed transplantation in SLE had inferior graft and recipient outcome compared with diabetic ESRD patients.[17] One important consideration regarding SLE is that it might create obstacle from getting CDC cross-match negative donor and render them on long waiting list[18] as in this case, which would have definite adverse prognostic impact on transplant outcome.[19-21] However, transplantation would be associated with lower mortality and better quality of life than remaining on dialysis.[1,21] He had 100 mismatch with the proposed donor. Even single HLA mismatch adversely affects the graft survival (4, 22-25)(Figure 3). Though in the modern era of immunosuppression, the role of HLA matching has become limited,(26) however it still has a definite role in reducing cumulative drug dose and drug related toxicities.[27]



Mismatches	Graft Survival (%) Cox 1 yr	Cox 5 yr	Kaplan–Meier 1 yr	Kaplan–Meier 5 yr
0	84.3	65.4	84.8	65.2
1	80.1	57.9	79.5	55.4
2	78.8	57.5	77.9	55.0
3	78.7	54.7	78.0	52.6
4	77.0	54.7	75.7	51.6
5	77.2	53.0	75.7	49.7
6	76.1	52.3	74.1	47.7
All	77.9	55.0	76.8	52.1

Figure-3: *Survival of First Cadaveric Renal Transplants in Patients with End-Stage Renal Disease, According to the Number of HLA-A, B, and DR Mismatches, 1984-1990.(25)[Adapted from NEJM 1994;331(12): 765-70.]*

"The patient had no DSA and Flow Cytometry Crossmatch (FCXM) was negative. However, complement dependent cytotoxicity crossmatch (CDCXM) was positive" - this needs in depth discussion.

Positive CDCXM was considered as a contraindication to transplantation before the availability of more specific and sensitive methods like FCXM and solid-phase assays. However in this case, the decision was not straightforward. Before coming to the conclusion, different crossmatch methods and their limitations should be discussed.

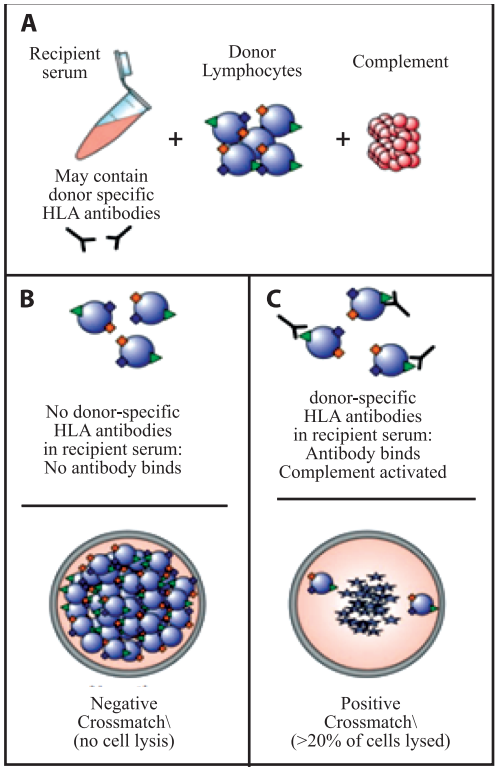
The idea behind the crossmatch is to detect the potential recipient who would likely to develop antibody mediated acute vascular rejection to a specific donor organ, thereby assess the suitability of transplantation between a specific donor and recipient pair. Graft loss from hyper-acute rejection due to presence of donor specific anti-HLA antibody was first described by Patel and Terasaki in 1969.(28) Following then crossmatch became a prerequisite for transplantation to prevent hyper-acute rejection.[29]

In crossmatch, serum from a potential recipient is tested for the presence of donor specific anti-HLA antibody to a potential donor. These antibodies can be against HLA I or HLA II or both. On the T cell surface, only HLA class I antigen is expressed whereas B cell expresses both HLA I and HLA II antigens on their surface. If the recipient has only class II anti DSA antibody, only B cell crossmatch will be positive. Whereas, presence of class I anti DSA antibody will result both T and B cell crossmatch to be positive.

Following introduction in 1960s by Terasaki,(28) there has been progressive improvement in this field to increase its sensitivity and specificity(30-32). Gebel et al. in their study of 703 sera in 2000, found that antihuman globulin enhanced cytotoxicity (AHG-CDC) based assay, enzyme-linked immunosorbent assay (ELISA) and microbead-based assay are progressively more sensitive by a factor of about 10% for detection of donor specific antibodies.(33) Karpinski M et al. in 2001 found Eighteen (13%) of 143 patients with negative T cell AHG-CDC crossmatch exhibited a positive retrospective T cell FCXM with significant adverse graft outcome.(30) Each of these methods is complementary to others.

Complement-dependent cytotoxic crossmatch (CDCXM)

In CDCXM, recipient's serum is incubated with donor lymphocyte,(34) then complement and vital dye are added. If recipient has anti DSA antibody it binds to the HLA antigen on T and B cells, complement is activated in classic pathway, membrane attack complex is generated which ultimately lyses the cells. Upon lysis, cells take up the vital dye and visualised under microscopy. By measuring the percentage of damaged cells strength of test positivity is assessed semi-quantitatively. Dead cell percentage of 0-10 is considered as negative(34)(figure 4, table 1). B and T cells are assessed separately.



The CDC crossmatch. recipient serum potentially containing donorspecific anti-HLA antibodies is added to donor T or B lymphocytes, along with complement (A) If donor-specific antibodies are not present, no lysis occurs and the result is deemed negative (B). If donor-specific anti HLA antibodies bind to the lyphocytes and then activate complement, cell lysis will occur and then activate complement, cell lysis will ocur andthe crossmatch result will be deemed positive (C) The proportion of lysed cells is assessed and the crossmatch isgraded a being weakly, moderately or strongly positive.

Figure-4: *CDCXM [adapted from Nephrology (Carlton) 2011;16(2):125-33]*

Table-I. Cytotoxicity scoring system (34)

Daed cells	Score	Interpretation
Not readable	0	Invalid technical issue)
11-20	2	Negative
21-50	4	Weak positive
51-80	6	Positive
> 80	8	Strong positive

[Adapted from Methods Mol Biol. 2013;1034:257-83.]

After implementation of CDC crossmatch, rate of hyper-acute rejection has been reduced dramatically, but still there was significant rejection that was indicating lack of sensitivity of this method.(28) Adding anti-human globulin (AHG) with this assay, that is, AHG-CDC increases the sensitivity significantly.(35)Kerman RH et al. in 1991 found recipients with positive AHG-CDC crossmatchwere associated with 36% 1 year allograft loss, whereas AHG-CDC crossmatch negative recipients had 18% graft loss.[36]

Interpretation

Positive T cell crossmatch indicates the presence of DSA against Class I antigen which has grave consequence on transplantation.[28] Patel and Terasaki in 1969 described the outcomes of 30 such transplants. Eighty percent lost their grafts immediately due to hyper-acute rejection while another 10% lost their grafts within 3 months.[28] Total grafts lost in cohort – 90%.

Isolated positive B cell crossmatch has a high chance of false positivity (around 50%).(37, 38) Negative result is reassuring as it indicates absence of both types of DSA. Positive B cell crossmatch correlates significantly only when simultaneously class II DSA is also detected by luminex technology.[39]

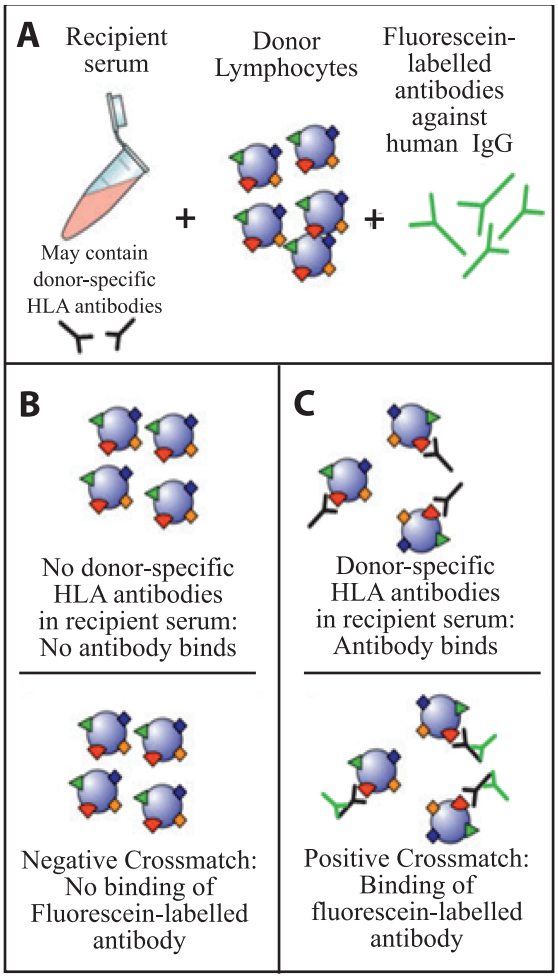
Positive T cell crossmatch in the setting of negative B cell crossmatch is usually considered as technical error.[40]

Limitations

Though introduction of CDC was a breakthrough in transplantation, it has some limitations. Due to low sensitivity, it cannot always detect the recipient with anti-HLA DSA. This results in false negative crossmatch, especially when the antigen for which the antibody is speciûc, is expressed only at very low levels on the donor's lymphocytes or when the antibody is of a type that does not activate complement and the most importantly, when the antibody titre is too low to cause complement activation.[40] It can also be falsely positive in the presence of non-HLA antibodies, autoantibodies and IgM type of antibodies which are considered to be non-pathogenic.[41-42] False positive crossmatch due to IgM autoantibody can be mitigated by adding-dithiothreitol(DTT) with recipient's sera and re-crossmatch. By reducing disulphide bond DTT will inactivate the IgM. If the positive crossmatch is due to IgM antibody, it will now be negative. In presence of autoantibody if recipient's sera is tested against recipients lymphocyte, the test will be positive,(40) which might be the fact in this case.

Flow CytometryCrossmatch Methods (FCXM)

To overcome the limitation of CDC crossmatchGarovoy MR et al. in 1983 introduced the Flow Cytometry-crossmatch (FCXM) system(43)(figure 5). The basic principle of FCXM is same as CDC crossmatch. Here



The flow crossmatch. recipient serum potentially containing donorspecific anti-HLA antibodies is added to donor t or B lymphocytes, along with fluorescein-labelled antibodies against humman Igg (A) If donor-specific antibodies are not present, no binding occurs and the result is deemed negative (B) If donor-specific anti-HLA antibodies bind to the lymphocytes these can then bind the fluoresceinlabelled antihukan IgG antibody, and this will be detectable by flow cytometry C). The strength of the fluorescence can be measured and expressed s 'channel shifts' above the control sample.

Figure-5: *FCXM [adapted from Nephrology (Carlton) 2011; 16(2):125-33]*

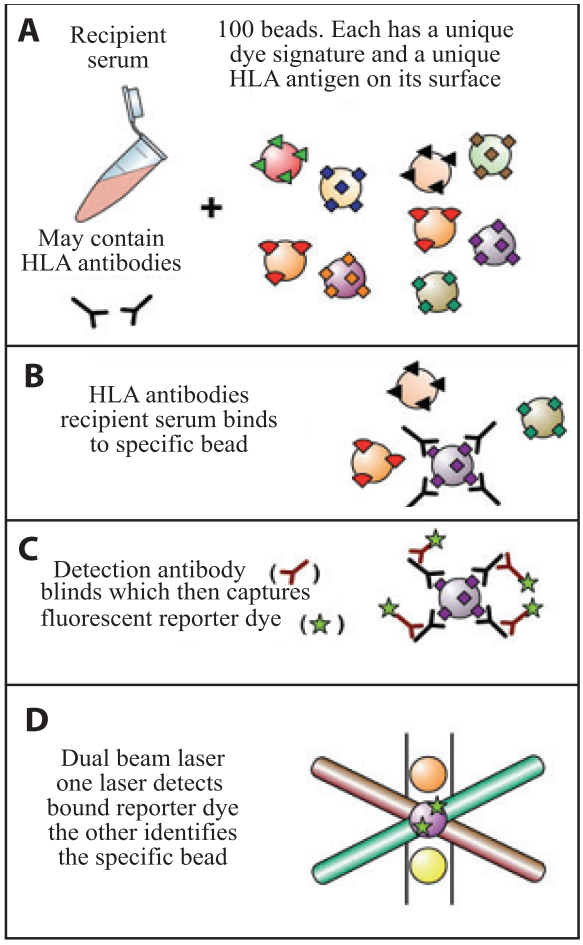
recipient's serum is incubated with donor lymphocyte and fluorochrome conjugated anti-IgG antibody is used for staining. FCXM detect both complement fixing and non-complement fixing DSA. Fluorochrome conjugated immunoglobulin binds to the donor specific antibody antigen complex on cell surface and is measured by flow cytometry.Additional antibodies with different fluorochromes that are specific to unique B and T cell antigen is used to differentiate them. FCXM can detect very low level of antibody which is otherwise missed by CDC crossmatch.(43) It is not reactive to IgM antibody.

The role of positive FCXM in CDC XM negative transplantation is not very clear. Increasing evidence suggest that it has a definite impact on transplant outcome. Christiaans and colleagues found no advantage of FCXM on transplant outcome in non-sensitised patients(44), whereas Limaye and colleagues found significant effect on long term graft survival in highly sensitised cases.[45]

The main limitation of FCXM is inter-laboratory variation of detection threshold.(32) The role of non-complement fixing DSA is yet to be established.

Virtual crossmatch

In virtual crossmatch (VXM), donor HLA antigen is compared with potential recipient's anti-HLA antibody specificity profile by computer based analysis system. Serum of every waitlist patient is periodically checked for DSA by Luminex-SAB. HLA antigen coated microspheres (beads) are used to detect the anti-HLA antibody in patient's sera (Figure: 6). When a donor is available, HLA typing is done immediately and compared it with the known antibody specificities of potential recipient. Recipient must have a recent antibody profile. Advantage of VXM over other crossmatching is that, it does not detect the non-HLA antibodies.[40,46] It is as sensitive as FCXM[47] and has a definite prognostic value in transplantation. Amico and colleagues prospectively studied 233 renal allograft recipients and concluded that VXM negative recipients had low risk of antibody mediated rejection (AMR) and early graft loss; whereas positive VXM with negative CDCXM recipients had significant risk of AMR despite intensified induction therapy.[48] VXM system also extended the scope of getting suitable donor for highly sensitised patients.[49]



The virtual crossmatch. Recipient serum potentially containing anti-HLA antibodies is added to a mixture of synthetic beads. Each bead is coated with a set of antigens (screening beads) or for more precise detail, with a single antigen (single antigen beads). A unique dye signature (up to 100) specifies the identity of each bead (A). If anti-HLA antibodies are present these will bind to the appropriate bead (B) ad detection antibody can subsequently bind and capture a reporter dye (C) Each uniue bead can then be interrogated for the presence of the reporter dye on its surface using a dual beam laser (D). A profile of antibodies can thus be identified in the recipient and compared with the known HAL identity of any potential donor, allowing a prediction of the crossmatch result.

Figure-6: *VXM [adapted from Nephrology (Carlton) 2011;16(2):125-33]*

Despite dramatic developments in crossmatch science, none should be used as standalone; one should be used complementary to other. Given case is a classic example of this.

Negative DSA in this case virtually excluded the presence of IgG anti-HLA antibodies (Table 2).

Table II. Causes of positive crossmatches – sorted by immunologic relevance(50)

Crossmatch type	Caused by	Supportive testing results
Immunologically RELEVANT positive crossmatches		
i and ɒ ceil	IgG class I HLA antibody	Solid phase testing will be positive for class I antibody
ɒ cen	**low uier class i** aHUDuUy	Solid phase testing positive for class I antibody
a cell	**Igu Class 11 HLA antibodv**	Solid phase testing positive for class II antibody
T and B cell or B cell alone	IgG class I and class II HLA antibody	Solid phase testing positive for both class I and class II antibody
Immunologically IRRELEVANT positive crossmatches		
T and/or B cell	Autoantibody	Autocrossmatch positive
T and/or B cell	IgG non-HLA antibody	Solid phase testing negative for class IorDHLA Ab
T and/or B cell (CDC or AHG CDC only)	IgM non-HLA antibody	Negative after DTT treatment of serum
T and/or B cell (CDC or AHG CDC only)	IgM class I or class II HLA antibody	Negative after DTT treatment of serum
T and B cell	Thymoglobulin/ Alemtuzumab	Clinical history of drug given
Bcell	Rituximab	Clinical history of drug given

[Adapted from Transplantation reviews (Orlando, Fla) 2009;23(2):80-93]

The patient might have non-HLA antibodies or autoantibodies which could not be detected by Luminex-SAB.[47] As FCXM was also negative which excluded the possibilities of non-HLA antibodies[40] Now two possibilities would need farther consideration- first "IgM antibodies" and second "other autoantibodies".

As both the above mentioned methods cannot identify IgM antibodies, there is strong possibility that the CDCXM positivity is due to the presence of IgM antibodies. This can be confirmed by treating the sera with DTT and re-crossmatch.[40] If still the test remained positive, it should be considered that, the presence of non-IgM autoantibodies which farther could be confirmed by mixing patients lymphocytes with his own serum. In presence of autoantibodies, the test would be positive. There are several factors which can cause formation of autoantibodies as well as false positive CDCXM; SLE is one of them.[51] In SLE, autoantibodies are of usually IgG type. Importantly transplantation in this type of patients is uneventful despite positive CDC crossmatch.[52]

Conclusion

In conclusion, my impression regarding this patients CDCXM positivity is due to autoantibodies associated with his primary disease, SLE. Though there are several arguments regarding outcome of transplantation in this case, considering his age, transplantation is still the best option, even with the given donor under augmented immunosuppression and post-transplant protocol biopsy.

Conflict of interest: None

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