Impact of the Virtual Crossmatch Assessment on Transplant Outcomes

Nadia Lewis BS, Carly J. Amato-Menker PhD, Ambreen Azhar MD, Gaurav Gupta MD, Mary Carmelle Philogene PhD
Virginia Commonwealth University, Richmond, VA, United States

Introduction

The virtual crossmatch (VXM) is an assessment of the immunological risk associated with a transplant that evaluates the patient's HLA antibody profile against the HLA typing of a potential donor. Several reports have shown the advantages of utilizing VXMs over prospective flow physical crossmatches (FXM), including cost-saving, shortened waitlist time, and reduced allograft cold ischemia time. Indeed, many programs will now proceed to transplantation following only a VXM; therefore, its accuracy must be routinely confirmed.

At VCU, a VXM is performed for each potential kidney and heart transplant candidate. If the VXM is deemed "acceptable," the transplant can proceed and will be followed by a retrospective FXM within 24 hours. If the VXM is deemed "unacceptable," a FXM must be performed prior to transplantation. In this study, we retrospectively assessed the VXMs for all renal transplant candidates that proceeded to transplant between August 3rd, 2023 and March 31st, 2024. VXM assessments were compared with FXM results and transplant outcomes, as evaluated by immediate graft function, AlloSure® cell-free DNA scores, and rejection events.

Methods

- 1114 VXMs were performed clinically for waitlisted renal patients for a total of 230 deceased donors during the study period, resulting in 126 transplants. Retrospective analyses assessed patient characteristics, correlation of VXM with FXM, and transplant outcomes at a median of 100 days follow-up.

- Antibody profiles established by Werfen single antigen with the following risk stratification:
  - Low risk – 1000-2000 MFI
  - Moderate risk – 2000-3000 MFI
  - High risk – >3000 MFI (>5000 MFI for C, DRB345, DQA, and DPA); repeat mismatches and cross-reactive antigens as needed
    - **listed as unacceptable in UNET**
  - DSA for unexpected positive FXMs were confirmed by One Lambda single antigen.
- VXM acceptable criteria:
  - Patient's serum in date (<60 days old)
  - Absence of DSA or presence of low risk DSA; no recent sensitizing events
  - Acceptable VXMs are followed by a retrospective FXM
- VXM unacceptable criteria:
  - Patient's serum out of date (>60 days old)
  - Moderate risk DSA present
  - cPRA ≥ 80 and/or sensitizing event has occurred since last antibody testing

- FXM were performed using the Halifaster protocol with a positive threshold set at ≥70 MCS for T and B cells. Lymphocytes were isolated from donor PBL, lymph nodes, or spleen using Stemcell EasySep cell isolation kits. Cells were incubated with patient serum in duplicate, then stained with CD3, CD19, and IgG-FITC, and acquired on FACSLyric (BD). MCS was calculated by subtracting the pooled negative serum MCFI from the patient's serum MCFI.

- Cell-free DNA scores were obtained via the AlloSure® assay (CareDx).

Results

Table 1. Demographics and outcomes of patients transplanted following a virtual crossmatch

Transplanted Patients			
	Total n = 126	VXM acceptable n = 111	VXM unacceptable n = 15
Demographics			
Sex (% female)	51 (40.5%)	42 (37.8%)	9 (60%)
Median age (range)	59.5 (14-77)	60 (14-77)	51 (30-74)
Race (% African American)	86 (68.3%)	76 (68.5%)	10 (66.7%)
Pre-formed DSA (% with DSA)	22 (17.5%)	17 (15.3%)	5 (33.3%)
Median follow-up time in days (range)	100 (1-238)	103 (1-238)	94 (19-211)
Positive T and/or B FXM (%)	6 (4.8%)	6 (5.4%)	0 (0%)
Transplant Outcomes			
Immediate Graft Function (%)	67 (53.2%)	60 (54.1%)	7 (46.7%)
Delayed Graft Function (%)	50 (39.7%)	48 (43.2%)	2 (13.3%)
Mean AlloSure® Score (range) at the end of follow up	0.41 (0.04-2.8)	0.41 (0.04-2.8)	0.38 (0.14-0.99)

Figure 1. Flow crossmatch results and antibody profiles of patients transplanted following an acceptable VXM

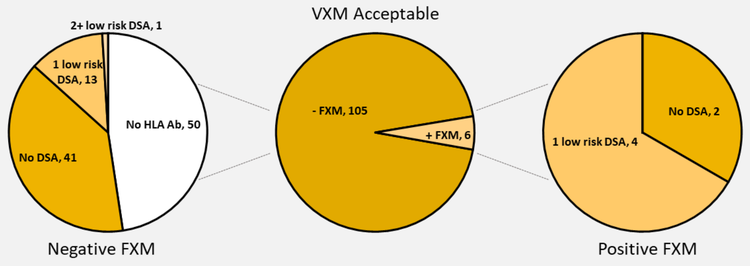

Table 2. Characteristics and clinical outcomes of patients with an unexpected positive FXM

Patient #	cPRA	Known sensitizing events	Pre-formed DSA	FXM MCS (T/B)	Post-tpl DSA	Clinical Outcome
1	56	Pregnancies and transfusions	None	56/78	De novo 3 months post-tpl	Renal vein thrombosis, graft nephrectomized
2	43	Pregnancies and transfusions	None	45/113	None	Immediate graft function; No rejection events
3	0	Pregnancies	Low risk DR8	0/85	De novo high risk class II 10 days post-tpl	Post-tpl desensitization d/t presumed subclinical AMR; graft function now stable
4	0	Stem cell transplant and transfusions	Low risk Bw4	94/98	Resolved	DGF; No rejection events
5	0	Pregnancies	Low risk B44	149/206	Resolved	DGF; No rejection events
6	7	Pregnancies and transfusions	Low risk A2	147/164	Resolved after desensitization	Post-tpl desensitization; No rejection events

Discussion

- The current protocol for VXM assessment resulted in a 95% accuracy with the FXM. Transplant outcomes were equally successful between VXM Acceptable and VXM Unacceptable patients that proceeded to transplant, as evaluated by % immediate graft function and AlloSure® cfDNA scores (p = 0.436).
- For each potential deceased donor, up to six patients are selected for a VXM assessment to ensure adequate backups if the primary recipient is incompatible or medically excluded. During the study period, 1114 VXMs were performed for a total of 230 deceased donors, resulting in 126 transplants. 111 of these transplants occurred following an Acceptable VXM, while 15 followed an Unacceptable VXM.
- Six transplant recipients with Acceptable VXMs had unexpected Positive FXMs.
  - Five of the six patients were female with possible undetected reactivity due to previous pregnancy.
  - One patient (Patient 1) experienced renal vein thrombosis and loss of the graft. The remaining five patients continue to have stable graft function.
  - One patient (Patient 2) was negative for HLA antibody by both single antigen panels, and did not develop DSA post-transplant. The Positive FXM was likely not driven by HLA.
  - Two patients (Patients 3&6) underwent post-transplant desensitization due to the Positive FXM, allowing for the resolution of DSA and/or presumed subclinical AMR.
- As reported in publications, a VXM may be difficult to predict in the presence of multiple low level DSA or epitope spreading such as the case with a Bw4 DSA (Patient 4).
- All VXM Unacceptable assessments resulted in a Negative FXM. Since this cohort consists of highly sensitized patients who received a transplant, it does not include sensitized cases who were not transplanted due to a positive FXM but does suggest that unacceptable antigens added to UNET were appropriately assigned.
- Delayed graft function (DGF) was observed in 43.2% of VXM Acceptable and 13.3% of VXM Unacceptable patients. This elevated rate is likely attributable to non-immunological factors and the increased proportion of African American patients and donors with additional risk factors. Patients with DGF did not demonstrate significantly higher AlloSure® scores (p = 0.098; data not shown).
- This study found that the VXM is an effective and time/cost-saving assessment of immunological risk. It also supports the continued use of retrospective physical FXMs to aid clinicians in post-transplant management.

Contact

•Contact: Mary.Philogene@vcuhealth.org

• Authors have no conflict of interest.

MAGNET RECOGNIZED
AMERICAN NURSES CREDENTIALING CENTER