Incremental Clinical Benefit and Incremental Cost Effectiveness Ratio of Routine Flow Crossmatch Prior To Kidney Transplantation

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INTRODUCTION

Rigorous molecular diagnostic testing using Next-Generation Sequencing (NGS) and Single Antigen Bead (SAB) methods to avoid HLA pre-sensitization dramatically reduces the rate of primary antibody-mediated rejection (AMR). Within this context, we examine the incremental value of routine pre-transplant flow cytometric cross-match (FCXM), often performed to further inform selection and management.

METHODS

Recipient and donor pairs considered for transplantation from 2016-2023 who had no historical or current DSA by SAB (threshold 1,000 MFI or lower based on epitope analysis), were then tested by FCXM and stratified by result. Patients with a +ve FCXM were reviewed to confirm their negative SAB history and were transplanted. All recipients were managed according to standard guidelines and followed throughout their course by a single specialist team. The incremental clinical benefit (ICB) of FCXM testing measured by time to biopsy confirmed acute rejection (BCAR), patient and graft survival was analyzed using Cox proportional hazards models and economic outcomes were compared using an incremental cost effectiveness ratio (ICER).

COHORT

Test cohort: A total of 2428 patients and 1687 donors were included in the current study. From these, 12,033 FCXM were performed on 7464 serum samples, each with a negative virtual crossmatch (VXM) against the potential kidney transplant donors. FCXM tests showed a high concordance with the negative VXM, and only 242 (2.01%) had a positive T- and 325 (2.70%) a positive B-FCXM.
Transplant cohort: Of all tested patients, a nested set of 1869 were transplanted with kidneys from 1333 donors. All had a negative VXM, though 34 (1.82%) had a positive T- and 48 (2.57%) had a positive B-FCXM against their specific donor.

	Tested	Transplanted
# Patients	2428	1869
# Samples	7464	1869
# Donors	1687	1333
# Patient - Donor Pairs	6668	1869
# Sample - Donor Pairs	12033	1869
# positive T-FCXM	242 (2.01 %)	34 (1.82 %)
# positive B-FCXM	325 (2.70 %)	48 (2.57 %)

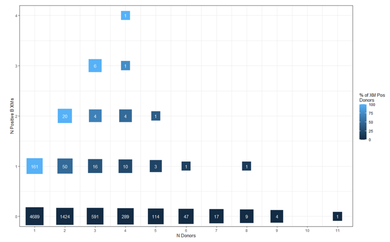
Patient -, sample - and donor numbers as well as FCXM positivity rates.

	Tested N = 2,428	Transplanted N = 1,869
Sex, n (%)		
Female	828 (37.3%)	632 (36.3%)
Male	1,391 (62.7%)	1,110 (63.7%)
Unknown	209	127
Race, n (%)		
Asian Indian	285 (11.7%)	210 (11.2%)
Asian Oriental	285 (11.7%)	226 (12.1%)
Filipino	209 (8.6%)	172 (9.2%)
White	1,062 (43.7%)	806 (43.1%)
Unknown	220 (9.1%)	137 (7.3%)
Other	367 (15.1%)	318 (17.0%)
Age		
Mean (SD)	60.71 (15.46)	54.61 (15.38)
Range	2.00 - 81.00	2.00 - 81.00
Unknown	212	127
Donor Status, n (%)		
Deceased	N/A	1,391 (74.4%)
Living	N/A	478 (25.6%)

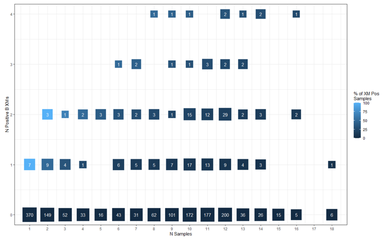
Patient demographics of the test- and transplant cohort.

RESULTS

In the test cohort, each sample was tested against up to 11 different donors while each donor served as the target for up to 18 recipient samples. Recipients with a positive FCXM despite a negative VXM demonstrated a range of patterns, in some only a single serum testing positive while in others almost all sera were consistently positive in the absence of demonstrable DSA.



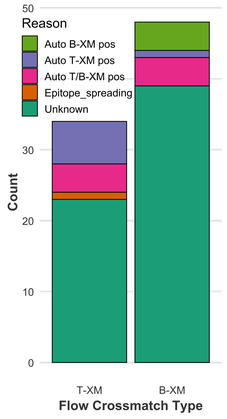
Serum samples were tested against a range of 1 to 11 donors each, with different positivity rates (B-FCXM shown).



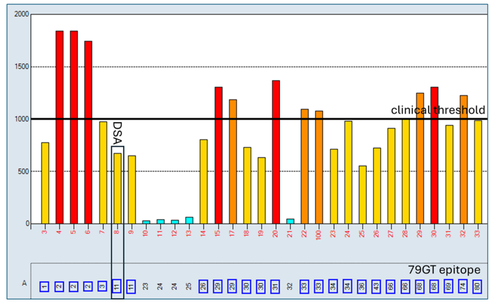
Donors were tested against a range of 1 to 18 different samples (some from the same patients), with different positivity rates (B-FCXM shown).

In the transplant cohort the reasons for a positive FCXM with a negative VXM were investigated further but not resolved in the majority of patients. Certain patients that consistently tested FXCM positive had a positive auto-FCXM associated with underlying pathology (e.g. autoimmunity, HIV) or therapeutic interventions (e.g. rituximab).

One sample with a positive T-FCXM was potentially explained by epitope spreading in which antibodies in the tested serum bind to multiple single antigen beads that share a common epitope (79GT), causing dilution of the binding and reducing the MFI below the clinical threshold, resulting in a negative VXM. For some other positive FCXM, donor-specific alleles were partially missing from the SAB test. However, due to the absence of other reactivities in those samples, this is unlikely to be the reason for the positive FCXM result.



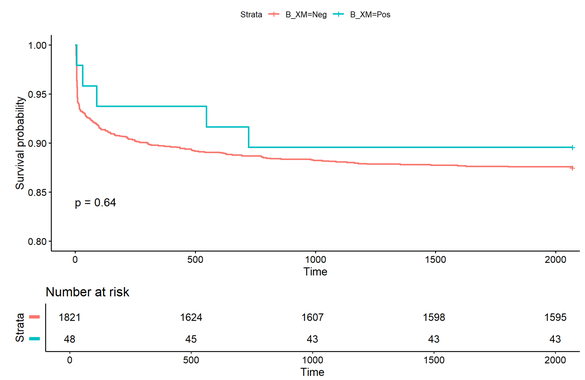
Reasons for unexplained positive FCXM results in transplant cohort. Auto-XM pos results could be due to pathology or therapeutics.



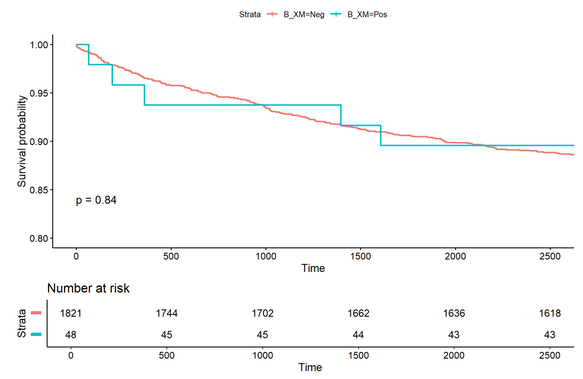
A negative VXM, which led to a positive T-FCXM most likely due to epitope spreading. Antibodies against the 79GT epitope, which is shared by many HLA-A antigens, were distributed across multiple single antigen beads, diluting the MFI of the donor-specific HLA-A*11:01 allele to below clinical threshold levels.

INCREMENTAL CLINICAL BENEFIT

During the 6 years of observation, 231 patients (12.3 %) developed biopsy-confirmed acute rejection (BCAR), 208 (11.1 %) lost their graft and 170 (9.1 %) died. Survival analysis using a Cox proportional hazards model incorporating cardinal baseline variables did not show any significant influence of pre-transplant FCXM on BCAR (T-FCXM: p=0.87 B-FCXM: p=0.64), graft loss (T-FCXM: p=0.95; B-FCXM: p=0.84) or death (T-FCXM: p=0.47; B-FCXM: p=0.37).



Unadjusted Kaplan-Meier of time to BCAR. Groups stratified by B-FCXM status.



Unadjusted Kaplan-Meier of time to graft failure. Groups stratified by B-FCXM status.

CONCLUSIONS

Precision molecular diagnostics have dramatically reduced the risk of primary AMR. While FCXM may inform decisions, routine pre-transplant testing confers **no additional predictive value or ICB**, with an ICER approaching infinity, assuming a recent SAB test, absence of interim sensitization, and inclusion of the DSA target on the SAB panel. This test should therefore be **used selectively rather than routinely** in renal transplantation.