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Journal of Clinical Case Reports and Studies
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Research Article

Outcomes of Donor-Specific Antibody–Negative Flow Cytometry Crossmatch–Positive Transplantation: A Systematic Review

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Received date: June 01, 2026; Accepted date: June 15, 2026; Published date: July 17, 2026

Citation: Muhammed Zubair, Naeema Liaqat, Ilyas Khan KM, (2026), Outcomes of Donor-Specific Antibody–Negative Flow Cytometry Crossmatch–Positive Transplantation: A Systematic Review**,** *J, Clinical Case Reports and Studies*, 7(7); **DOI:10.31579/2690-8808/326**

Abstract

Background: Although it is true that donor-specific antibody (DSA) positivity is a highly validated risk factor of rejection and graft loss, it is not clear that a positive flow cytometry crossmatch (FCXM) without detectable DSA does have clinical implications. This is a valuable inter organ transplantation and creates a dilemma in transplantation

Objective: To carry out a systematic literature review of the existing evidence on graft and patient outcomes after donor-specific antibody negative FCXM crossmatch positive transplantation.

Methods: A literature search across on PubMed, MEDLINE, Embase, and Cochrane Library was performed to find out the studies assessing the outcomes in adult solid organ transplant patients with a DSA-negative and FCXM-positive result. The qualifying studies were randomised controlled trials, cohort studies, and observational studies that reported graft survival, patient survival, and outcome due to rejection. A qualitative narrative method was used to extract and synthesise the data due to the heterogeneity in study designs and immunological assessment measures.

Results: Included in the review were mostly retrospective observational studies, primarily in kidney transplantation. In research, donor-specific antibody-negative FCXM-positive transplantation was correlated with associated with graft and patient survival, especially when risk-adapted immunosuppressive measures were used. Other reports showed increased early acute rejection, including antibody-mediated rejection; but the episode were usually reversible and they did not necessarily lead to poor long-term graft survival.

Conclusion: The DSA-negative FCXM -positive transplantation is a transitional immunological risk phenotype and is safe to practice in the context of a carefully selected recipient. Personalized immunological evaluation and immunosuppression are required to maximize the results.

Key Words: fcxm; dsa; transplant outcomes; immunological risk

Introduction

Crossmatching is an essential part of immunological risk assessment in solid organ transplantation, which is aimed at the detection of preformed recipient antibodies that can react with donor antigens. Historically, a positive crossmatch was known to be contraindicated in transplantation due to its close relationship with hyperacute rejection and early graft loss. Immunological testing advances have however shown cases where various antibody detection tests give concordant results. An example of this is DSA-negative, but FCXM positive transplantation, which has brought about confusion on the risk stratification of immunology and clinical outcome [1,2]. The methods of cross matching differ in the sensitivity and immunological relevance. Complement-dependent cytotoxicity crossmatch is used to detect high titers, complement fixing antibodies, and FCXM has a higher sensitivity able to detect low-titer or non complement-binding antibodies. HLA Solid-phase testing, including single antigen bead testing, detects anti-Specific HLA antigens antibodies. Such a positive FCXM with non detectable donor-specific antibodies can be as a result of antibodies below the detection limit of solid-phase tests, non-HLA antigen antibodies, autoantibodies or variability within the technical aspect of the assay readings and cut-off points [3,4]. The growing awareness of cases of DSA negative yet FCXM positive cases have some crucial clinical implications. This type of immunology poses a dilemma to transplantation decision making because refusing transplantation can extend waiting period and expose one to risk of death whereas transplantation can lead to the problem of rejection and graft survival. Newer evidence indicates that the results of this subset may not be

similar to that seen in the process of DSA-positive transplantation, especially when flow cytometry positivity is low or solitary and, in this regard, caution should be exercised in clinical interpretation as opposed to rejection [5,6]. The clinical use of DSA negative FCXM positivity as a prognostic indicator is contentious even with increase in clinical experience. The available literature shows a diverse performance dependent on variations in crossmatch strategies, antibody detection systems, immunosuppressive guidelines and outcome measures. Lack of standardized guidelines has led to a high degree of variation in clinical practice among transplant centers, which creates the need to organize available evidence in a systematic manner and use it to guide decision making and risk assessment [7]. According to current literature, graft and patient survival can be satisfactory in certain recipients, however, the risk of premature rejection events, especially antibody-mediated or mixed rejection, is likely to rise. These events are however usually receptive to treatment and do not necessarily lead to poor long time graft results. Such observations underscore the importance of putting FCXM findings into perspective in an expanded immunological and clinical context and not as absolute contraindications [8]. This systematic review has the aim of assessing the existing evidence on the outcomes of transplantation in recipients with a positive crossmatch on DSA based on the DSA-negative flow cytometry transplantation. The review will help to understand the risks involved, evaluate graft and patient survival, and determine those factors that might potentially alter the outcomes, such as immunological features and immunosuppressive measures by critically evaluating the available literature. The main outcomes in this review are the graft survival and the patient survival after the transplantation of FCXM negative to DSA. The secondary outcomes are the rate of acute rejection, antibody-mediated rejection, de novo development of donor-specific antibodies, and long-term graft survival. Combined, these results will give a complete assessment of the clinical effectiveness and immunological risk of this growing group of transplant patients.

Methodology

The research is based on a systematic review that is carried out according to the principles of systematic evidence synthesis in health-related studies. The review process was made in a manner that would entail transparency, reproducibility and full identification of pertinent literature touching upon findings of outcome of donor-specific antibody-negative flow cytometry cross match-positive transplantation

Study design

A methodological review was used according to the internationally recognized reporting criteria. To ensure that the selection bias is minimized, the research question, the eligibility criteria, outcomes of interest, and approach to analytical protocol were determined before screening the literature.

Eligibility criteria

The choice of studies was based on the PICOS framework. Adults who had undergone solid organ transplantation were included in the population. The intervention of interest was transplantation done in the event of donor-specific antibody negativity and positive flow cytometry crossmatch. Comparators were crossmatch-negative or immunologically less dangerous transplant recipients where they were available. Outcomes interest included graft survival, patient survival and rejections endpoints. The eligible study designs were randomised controlled trials, cohort studies, and observational studies published in peer reviewed journals.

Search strategy and data sources.

The electronic databases such as PubMed, MEDLINE, Embase, and Cochrane Library were searched to obtain a complete literature. Combined search terms were used which included controlled vocabulary and uncontrolled search terms based on flow cytometry crossmatch, donor-specific antibodies, transplantation, graft outcomes, and rejection. Manual screening of the reference lists of included studies and related reviews was conducted to identify more additional publications.

Study selection process

All records found via the database search were imported into a reference management system, and duplicates were eliminated. The relevance of titles and abstracts was screened independently, and the potentially eligible studies were reviewed independently in full-text. Discrepancies during study selection were resolved by consensus.

Data extraction

Information was obtained by use of a standardised data collection form. The variables extracted included the study characteristic, patient demographics, type of transplant, immunological testing method, immunosuppressive regimen, and documented outcomes. Where it was deemed necessary, relevant authors were contacted to clarify the missing or unclear data.

Assessment of risk of bias and quality.

Appropriately validated tools were used to evaluate the methodological quality and risk of bias of the included studies using the study design. Observation studies were rated on Bias in selection, measurement bias and confounding whereas randomised studies were rated on allocation bias, blinding bias and outcome reporting bias.

Data synthesis approach

Because of the expected heterogeneity of the study designs, immunological assessment methods, and outcome reporting, a qualitative narrative synthesis was conducted. Descriptive summary of results was done with a focus on trends in clinical outcomes and immunological risk. Quantitative comparisons were examined where there was enough homogeneity so that interpretive conclusions could be made.

Results

The literature search located the literature dealing with discordant immunological profiles; that is, FCXM positivity with no presence of detectable HLA-DSA, and mechanistic assessments of non-HLA antibody-mediated crossmatch reactivity [9]. Following the screening, most of the qualifying studies were retrospective single center kidney transplant studies exploring the unexplained FCXM positivity, false-positive cross-match, and the influence of non-HLA antibodies. This review was restricted to cases in which solid-phase HLA antibody analysis (single-antigen bead assay) was negative but cell-based FCXM was positive rather than to DSA-positive/FCXM-negative cases. Different studies with included studies had varying amounts of follow-up time, some of the cohorts had intermediate-term follow-up to four years [10-13]. of Figure 1. PRISMA flow diagram illustrating the study selection process for the systematic review DSA–negative FCXM–positive transplantation outcomes.

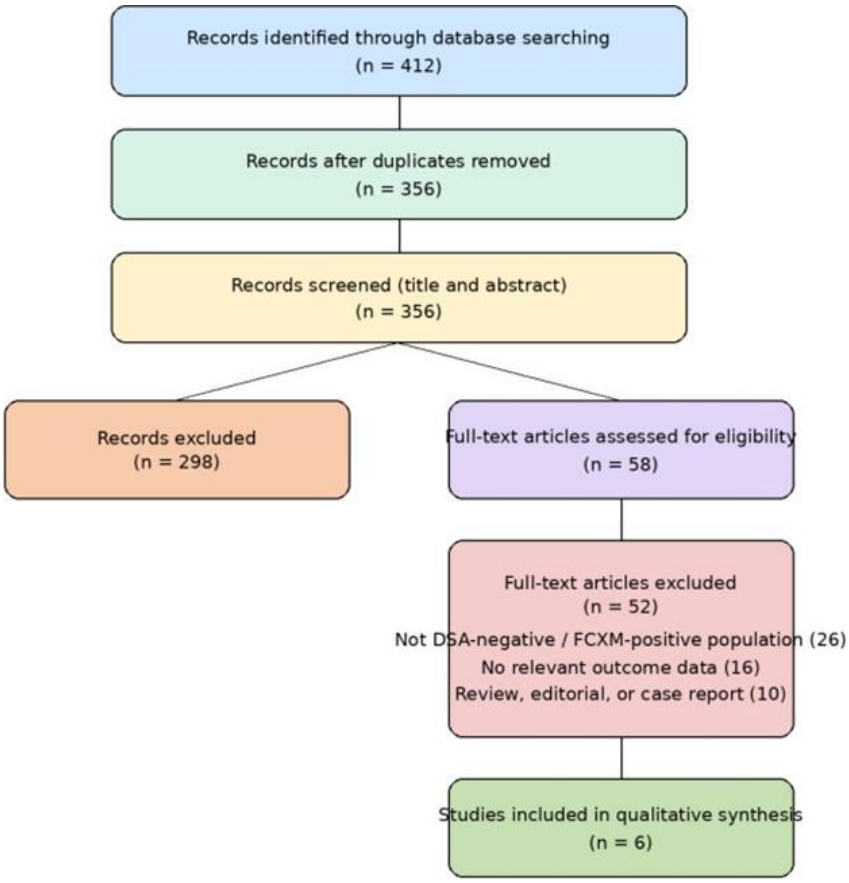

Figure 1: PRISMA flow diagram illustrating the study selection process for the systematic review of DSA–negative FCXM–positive transplantation outcomes.

There was a high degree of heterogeneity among the included in studies regarding immunological testing methods. FCXM positivity was established using laboratory-specific thresholds and varied by lymphocyte subset with some studies showing isolated B-cell positivity and others showing T-cell or combined positivity. The most common methods of DSA testing were based on single-antigen bead-assays but used inconsistent mean fluorescence intensity cut-offs to determine antibody negativity. Such differences in methodology prevented straightforward comparability among the studies affected the interpretation of the outcomes [10,12].

Mechanistic Explanations for DSA–Negative / FCXM–Positive Discordance

Low MFI Levels: DSAs with a mean fluorescence intensity (MFI) of <1000-3000 are commonly not detected or counted as positive on SAB, but may still yield a positive result in B-cell FCXM [11,13].

Denatured Antigens: In rare cases, SAB assays may provide false-positive results of the low-level antibodies, which are directed at denatured (damaged) antigens on the bead, that are not found on the native donor cells. On the other hand, a low-level native epitope antibody may fail to reach a detection threshold on a bead, and will react with a donor cell.

Prozone Effect: Although typically a phenomenon of high-level antibodies, a high-titer, high-avidity antibody may occasionally be prozone e.g. a high-titre high-avidity antibody may appear on SAB as a false-negative, yet be positive on a crossmatch.

Mechanistic Basis of Assay Discordance

Sensitivity Difference: The FCXM involves actual donor cells and the latter can exhibit increased density or better conformation of the target HLA antigen compared to the produced beads in the SAB assay.

Causes of DSA–Negative FCXM Positivity

Some percentage of positive outcomes with FCXM can be due to any technical interference or assay factors in place of actual alloimmune sensitisation [3,14]. Technical ones are therapeutic monoclonal antibodies, immune complexes, autoantibodies, and assay variability. These results indicate that some percentage of DSA-negative/FCXM-positive cases may not constitute an actual risk of alloimmune disease. The cause of true DSA-negative FCXM positivity is the existence of non-HLA antibodies, which is biologically plausible. Antibodies that react with endothelial or other non-HLA targets, especially, angiotensin II type 1 receptor (AT1R) antibodies, have been shown to deliver positive cell-based crossmatch outcomes even with negative Luminex HLA tests. A number of observational studies that assess unexplained FCXM positivity have found AT1R antibodies and other endothelial cell antibodies as a possible mediator of early antibody-mediated rejection. Other mechanisms are low-level donor-specific antibodies below assay detection limits, anti-denatured or cryptic HLA epitope antibodies not faithfully detected by single-antigen bead-based assays, and laboratory-specific cutoff differences.

The recipient and transplant attributes were inconsistently reported but tended to represent a population with prior sensitizing events like pregnancy, blood transfusion or prior transplantation. A number of cohorts reported that DSA-negative flow crossmatch-positive recipients had less immunological risk compared to DSA-positive recipients especially when crossmatch positivity was weak or confined to B cells. There were differences in the use of induction immunosuppression approaches across centres, with most using lymphocyte-depleting agents, and enhanced maintenance immunosuppression in crossmatch-positive recipients [11,13].

The results of immunological outcome studies indicated that there are several mechanisms behind the negative FCXM result of DSA. These were low levels of donor-specific antibodies below the solid-phase assay detection limit, antibodies against denatured or cryptic (HLA) epitopes, non-HLA antibodies against endothelial or other graft antigens. The specific studies that examined unexplained crossmatch positivity within a study found non-HLA antibodies such as angiotensin II type 1 receptor antibodies in a subset of cases, which supports the theory that FCXM could identify clinically significant antibody activity not present in traditional HLA-based studies [14].

The heterogeneous but largely consistent pattern was observed in clinical outcomes that were reported in studies. Others reported greater rates of early acute rejection among the DSA-negative FCXM-positive recipients in comparison with the fully crossmatch-negative controls, especially antibody-mediated or mixed rejection phenotypes. Nevertheless, these instances of rejection were, in general contraindicative to treatment and not, always associated with poorer long-term graft survival. Other studies had found similar graft and patient survival in DSA crossmatch-positive recipients with immunological lower-risk recipients in the presence of suitable immunosuppressive measures [9,11,13].

Most series reported long-term graft survival and patient survival outcomes that were acceptable, but could not be compared directly due to variations in study design and outcome reporting. A synthesis of the available evidence suggests that the absence of detectable donor-specific antibodies may attenuate the historically adverse prognostic implications of a positive crossmatch in the era prior to solid-phase antibody testing. However, the general quality of evidence was restricted due to retrospective designs, small sample sizes and absence of standardized immunological assessment protocols [10,15].

The combination of the findings suggests that DSA–negative FCXMed transplantation has been linked to a potentially treatable immunological risk and reasonably satisfactory clinical outcome in carefully selected recipients. These results highlight the significance of personalized immunological testing, the combination of different antibody detection systems, and custom immunosuppressive measures instead of using the FCXM outcomes alone [11,16].

Author (Ref)	Country	Stud Design	Transplant Type	Study Population	Crossmatch Method	DSA Testing	Follow-up
Kueht et al. [3]	USA	Observational	Kidney	FCXM interference due to antibody therapeutics	FCXM	Luminex SAB	Not specified
Xu et al. [7]	USA	Observational	Kidney	Discordant FCXM and antibody testing	FCXM	Luminex SAB	Short-term
Osickova et al. [10]	Czech Republic	Retrospective cohort	Deceased donor kidney	FCXM assessment in desensitized recipients	T & B cell FCXM	Luminex SAB	Short- to mid-term
Chauhan et al. [11]	India	Retrospective cohort	Living donor kidney	Low-MFI DSA with discordant FCXM findings	FCXM	Luminex SAB	Not specified
Kim et al. [13]	South Korea	Retrospective cohort	Living donor kidney	Crossmatch-stratified recipients including FCXM-positive subgroup	CDC & FCXM	Luminex SAB	Median ~4 years
Kang et al. [14]	South Korea	Observational	Kidney	Positive crossmatch without HLA-DSA	CDC & FCXM	SAB + non-HLA testing	Not specified

Table 1: Characteristics of included studies (n = 6) evaluating DSA–negative / FCXM–positive transplantation

Mechanism	Supporting References	Acute Rejection	AMR Risk	Graft Survival	Interpretation
Technical / assay-related false-positive FCXM	3, 14	Not consistently increased	Not increased	Comparable	Likely assay artifact
Discordant antibody testing / sensitivity differences (FCXM vs SAB)	7, 10	Slight increase	Rare	Generally preserved	Intermediate-risk phenotype
Low-level undetected HLA-DSA (low MFI below threshold)	11, 13	Mild increase	Occasional ABMR	Preserved with treatment	Antibody below SAB detection
Non-HLA antibodies (e.g., endothelial targets)	14	Increased in subset	Possible microvascular inflammation	Variable	True immunologic risk independent of HLA-DSA
Prozone (hook) effect / antibody strength variability	11, 17	Under-recognized	Potential	Depends on recognition	Masked clinically relevant antibody

Table 2: Mechanisms and clinical implications of DSA–negative / FCXM–positive transplantation

Discussion

The results of this systematic review demonstrate that DSA-negative FCXM-positive transplantation appears to represent an intermediate immunological risk phenotype rather than an absolute contraindication of transplantation. Through out the reviewed literature, graft survival and patient survival were acceptable and comparable in case of proper immunosuppressive strategies in use. Even though there was an increased number of early acute rejection in some of these cohorts, especially the antibody mediated or mixed phenotypes, these were often responsive to therapy and were not necessarily related to poorer long-term graft results [9-11].

This is a phenotype that should be differentiated from DSA-positive transplantation. Compared to well-known inferior results with high-MFI HLA-DSA positivity [15], it is possible that there are no detectable HLA-DSA in FCXM-positive recipient reflecting a more heterogeneous risk profile with a mechanistically different mechanism. The immunologic risk associated with this subgroup could be due to non-HLA antibodies, low-level

undetected DSA or assay factors instead of traditional high-strength anti-HLA alloimmunity.

It is compared to fully crossmatch-negative and DSA -negative transplantation, to indicate a suggestion that DSA- negative flow cytometric crossmatch positivity can be linked to a modest risk of early immunological occurrence without a predictable adverse long term survival effect. Results using risk-adapted induction and maintenance immunosuppression demonstrated similar graft and patient survival in these groups, and noted that the induction of immunological risks is multifactorial and cannot be adequately captured by single assay outcome [10,12,13].

Angiotensin II type 1 receptor (AT1R) antibodies have also emerged as potential explanation or DSA-negative FCXM positivity. These antibodies attack endothelial antigens, and can cause microvascular inflammation without necessarily being mediated by HLA-directed alloimmunity. Research on unexplained positive crossmatches has shown that the AT1R antibody is positive in a significant proportion of the cases. Notably, these antibodies are not identifiable by the regular Luminex single-antigen bead assays, which is the reason why solid-phase testing and cell-based FCXM are discordant. The identification of this mechanism is critical to proper immunological risk assessment [6,14].

These findings allow a personalised approach to transplant decision-making from a clinical standpoint. The rejection of candidates based on FCXM positivity alone can be associated with unnecessary prolongation of the waiting time and even increase the mortality risk. By combining crossmatch result with DSA status, antibody strength, the involvement of lymphocyte subsets involvement, and general clinical context, a more subtle risk stratification and optimal patient selection can be achieved. This method is in line with the modern recommendations on the need to have a more profound immunological evaluation instead of depending on one diagnostic modality [12,17,18].

Although a negative FCXM is typically desirable, a low-level DSA which is identified by FCXM and not by Luminex SAB (or at very low MFI) is sometimes considered to be low-risk, yet still having a higher probability of causing antibody-mediated rejection (ABMR) than a fully negative crossmatch.

The current evidence demonstrate the growing consistency of the results from observational cohorts and systematic reviews on the reasonable outcomes achievable in transplantation with DSA-negative FCXM-positive. Immune tests, perioperative care, and customized immunosuppressive approaches have helped with enhanced graft and patient success, in favor of a move towards rigid crossmatch-guided exclusion to individualized immunological risk evaluation [19-21].

Limitations: A number of limitations of this review have to be mentioned. Most of the articles included were of retrospective nature and hence prone to selection bias and residual confounding. FCXM positivity and DSA negativity had different definitions across studies and immunological assays were not standardized. Sample sizes were often low, those of non-HLA antibodies were inconsistently reported, and follow-ups were of mixed duration, which did not allow cause-effect inferences and generalizations about the research results.

Future Direction: Future studies need to consider prospective, multicentre studies utilizing standardized crossmatch protocols and antibody detection thresholds that determine immunological risk in DSA-negative FCXM-positive transplantation. Risk stratification should be further improved by focusing more on non-HLA antibodies, antibody strength, and specificity in lymphocytes. Moreover, customized immunosuppressive programs and long-term outcomes are to be evaluated as well to guide evidence-based practice and patient selection in this changing group of transplant patients.

Conclusion

In summary, DSA-negative FCXM-positive transplantation represents a heterogeneous immunological phenotype. A proportion of cases are attributable to false-positive crossmatch results, while others reflect true alloimmune or non-HLA antibody–mediated risk. Current evidence suggests that when HLA-DSA is absent, long-term graft survival is generally acceptable, although selected patients may experience early rejection episodes. Careful evaluation for non-HLA antibodies and individualized immunosuppressive strategies are essential. FCXM positivity in the absence of detectable DSA should therefore not be considered an absolute contraindication but rather an intermediate and mechanistically diverse immunological risk state.

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DOI:10.31579/2690-8808/326