Exploring The Role of Non-HLA Antibodies in Kidney Transplant Outcomes: Delayed Graft Function and Rejection

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Introduction

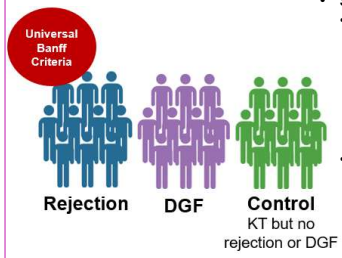
- *End-stage kidney disease* (ESKD) is a critical condition affecting over 45,000 Canadians. While kidney transplantation is the most effective treatment, post-transplant complications such as *delayed graft function* (DGF) and rejection continue to pose significant challenges.
- **Non-HLA Antibodies** - A New Focus: Traditionally, transplant complications have been linked to *human leukocyte antigen* (HLA) *donor-specific antibodies* (DSAs), but emerging research suggests that non-HLA antibodies may play a pivotal role in both DGF and rejection. These antibodies, which are exposed after tissue damage, may be key contributors to immune-related graft injury.
- **Impact of Non-HLA Antibodies on DGF and Rejection**: Non-HLA antibodies have been implicated in worsening ischemia-reperfusion injury, leading to inflammation and DGF—a complication that hinders early graft recovery and can negatively affect long-term survival. Additionally, these antibodies may trigger immune responses that cause graft rejection, even in the absence of detectable HLA DSAs.
- **Challenges in Non-HLA Antibody Research**: Despite their potential significance, the role of non-HLA antibodies in transplant outcomes remains underexplored due to non-standardized detection methods used in previous studies. This lack of uniformity has made it difficult to fully understand their impact on DGF and rejection.
- **Advancing Non-HLA Research**: Standardizing non-HLA antibody detection and integrating these findings into personalized immunosuppressive strategies could enhance post-transplant care. By focusing on non-HLA immunology, we can significantly improve the management of DGF and rejection, enhancing both short- and long-term outcomes for kidney transplant recipients.

Materials & Methods

- This Research Ethics Board (REB) approved study integrates both **retrospective** and **prospective** cohorts to assess the role of non-HLA antibodies in kidney transplant outcomes, focusing on DGF and rejection.

Retrospective Cohort:

- **Study Population:**



- **Sample Collection:**
  - Rejection and Control Groups: Blood samples collected pre-transplant and post-transplant from the time of transplant until the time of biopsy. Biopsy is mandatory for inclusion.
  - DGF Group: Post-transplant samples collected within 7 days of transplantation.

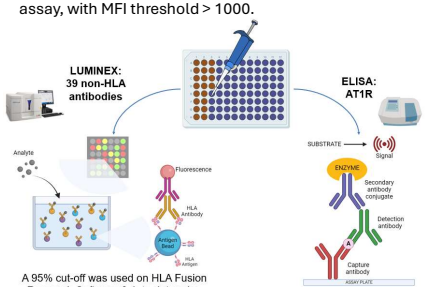
Clinical data on DGF, rejection (biopsy-confirmed), and graft outcomes sourced from medical records.

Prospective Cohort:

- **Study Population:** Ongoing recruitment of 400 healthy volunteers, with samples collected as participants volunteer (no transplant history).
- **Sample Collection:** Blood samples collected from healthy volunteers during study participation (no predefined time points).
- **Clinical Data:** Data collection includes general health information from healthy volunteers, focusing on immune profiles.

Antibody Testing:

- ***Non-HLA Antibodies***:
  - Luminex Assay: Detects 39 non-HLA antibodies.
  - ELISA (AT1R): Measures anti-angiotensin II type 1 receptor (AT1R) antibodies.
- ***HLA DSAs***: Assessed using Luminex single-antigen bead assay, with MFI threshold > 1000.



A 95% cut-off was used on HLA Fusion Research Software 6.4. to determine antibody positivity (1000 MFI).

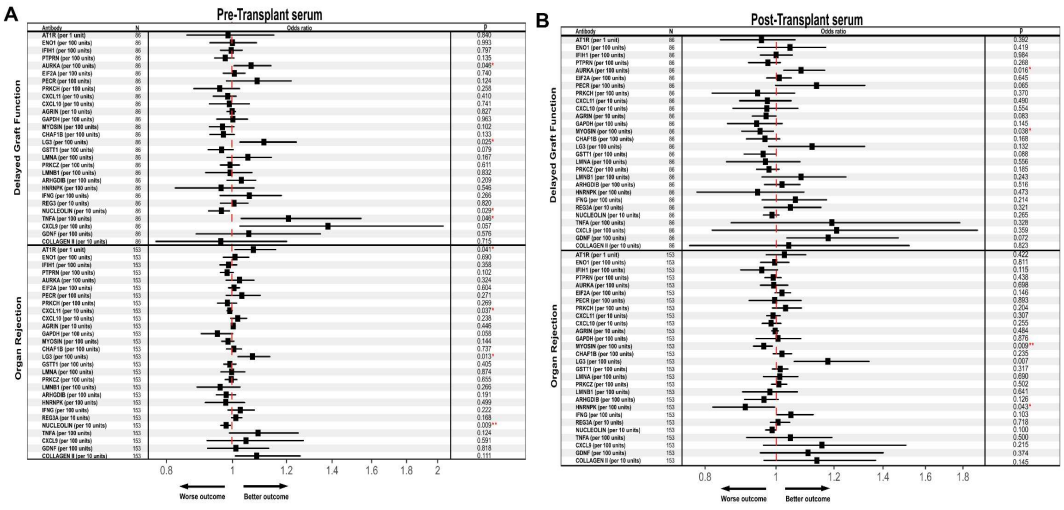
Readings ≥ 17 U/mL were defined as positive.

Statistical Analysis:

- Correlation and regression analyses were performed to investigate relationships between non-HLA antibody levels, HLA DSAs, and clinical outcomes in the retrospective cohort.

Results

Figure 1. Forest plots illustrating the odds ratios (OR) with 90% confidence intervals (CI) for non-HLA antibodies in relation to DGF and organ rejection. **(A)** Plot depicts odds ratios corresponding to an increase in normalized fluorescence units in pre-transplant serum samples measured by ELISA and Luminex 200, while **(B)** plot depicts odds ratios for post-transplant serum samples. Odds ratios for each plot were derived from multinomial logistic regression models adjusted for number of HLA DSAs and patient age at transplant. The reference line at OR=1 indicates no effect. Biomarkers to the right of the line are associated with increased odds of the outcome, whereas those to the left are associated with decreased odds. p-values are shown on the right of each odds ratio.



Conclusions

- Our findings highlight the potential role of non-HLA antibodies in adverse kidney transplant outcomes, such as delayed graft function (DGF) and rejection. While these antibodies show promise as significant contributors, it is crucial to account for additional variables—such as HLA DSAs, patient demographics, and immunological factors—that may influence transplant success.
- Preliminary analysis suggests meaningful correlations between post-transplant non-HLA antibodies, HLA DSAs, patient age, and overall transplant outcomes. However, further research is necessary to establish stronger evidence supporting these relationships and their clinical implications.
- By recognizing non-HLA antibodies as critical factors in transplant immunology, we open the possibility for their inclusion in routine diagnostic protocols, improving risk assessment, patient monitoring, and early rejection detection. Ultimately, this could guide the development of targeted therapies aimed at enhancing graft survival and optimizing post-transplant care.

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