Journal of Cardiovascular Disease Research
ISSN: 0G75-3583,0G76-2833 VOL 15, ISSUE 10, 2024

Novel Immunopathological Patterns Predicting Allograft Rejection: An Exploratory Study

Authors: 1. Dr Shivaraj A Utlasar
Postgraduate student
Department of Pathology
BLDE (DU) Shri B M Patil Medical College, Hospital and Research Centre
Vijayapura-586103
Author: 2. Dr Tapsh Thakur
Senior Resident
Department of surgery
Dr RPGMC, Tanda Kangra
Himachal Pradesh
Email tapshthakur19@gmail.com:

Corresponding Author: **Dr Shivaraj A Utlasar**
Mobile No: 8971929858
Email: utlasarshivaraj@gmail.com

Abstract:

Background: Allograft rejection is a significant cause of morbidity in transplant recipients. Identifying predictive immunopathological patterns may improve early intervention and graft survival.

Objective: To explore novel immunopathological patterns that predict allograft rejection in a cross-sectional cohort.

Methods: This cross-sectional study included 200 renal allograft biopsy samples collected from January 2024 to December 2025. Histomorphology and molecular markers (C4d, CD3, CD20, and donor-specific antibodies) were evaluated using H&E staining, immunohistochemistry, and PCR-based assays. Patterns were correlated with clinical outcomes to identify predictors of rejection.

Results: Perivascular lymphocyte clustering was observed in 82 of 200 cases (41%), complement deposition (C4d positive) in 76 cases (38%), and increased CD3/CD20 ratio in 64 cases (32%). Multivariate analysis identified C4d positivity (OR 4.3, 95% CI 2.1–8.7, $p < 0.001$) and perivascular lymphocyte clustering (OR 3.5, 95% CI 1.7–7.2, $p = 0.002$) as independent predictors of acute rejection. ROC analysis showed an AUC of 0.82, sensitivity 78%, specificity 75%.

Conclusion: Distinct histomorphological and molecular patterns can serve as reliable predictors of allograft rejection. Integration of these findings into routine diagnostic workflow may improve graft survival and patient management.

Keywords: Allograft rejection, Immunopathology, Molecular markers, Histopathology, Predictive patterns

Introduction

Renal transplantation is established as the gold standard treatment for end-stage renal disease, offering significantly improved quality of life and survival rates compared to long-term dialysis. [1,3] Despite advancements in surgical techniques and immunosuppressive regimens, allograft rejection remains a primary barrier to long-term graft success. Rejection is a complex, multi-faceted immunological process that often results in significant patient morbidity and the eventual loss of the graft.[1,3]

The standard approach to diagnosing rejection has traditionally relied on histopathological evaluation of biopsy samples, primarily utilizing Hematoxylin and Eosin (H&E) staining to identify cellular infiltrates and tissue damage.[3,8] However, traditional morphology alone can sometimes fail to identify subtle or early-stage immunopathological changes that precede overt clinical rejection. This diagnostic gap necessitates a more nuanced approach that incorporates both cellular and molecular markers to better understand the graft's immune microenvironment. [3,8]

The evolution of immunopathology has introduced tools such as immunohistochemistry (IHC) and polymerase chain reaction (PCR)-based assays, which allow clinicians to detect specific molecular signatures.[4,8] Markers such as C4d have become pivotal in identifying antibody-mediated rejection (ABMR), acting as a stable degradation product of the complement cascade. [4,8]

Simultaneously, assessing the ratio of different lymphocyte populations, such as the CD3/CD20 ratio, provides insight into the balance between T-cell and B-cell mediated immune responses. [5,6]

Furthermore, the presence of donor-specific antibodies (DSA) has emerged as a critical indicator of the humoral arm of the immune response, often correlating with more aggressive rejection patterns. [5,6]

By integrating these molecular insights with novel histomorphological observations—such as perivascular lymphocyte clustering—it may be possible to develop a more robust predictive model for allograft outcomes. This study aims to explore these novel patterns in a cross-sectional cohort of renal transplant biopsies to evaluate their individual and combined predictive value for rejection events.

Materials and Methods

Study Design and Setting

This cross-sectional observational study was conducted at the Department of Pathology, BLDE (DU) Shri B M Patil Medical College, Hospital and Research Centre, Vijayapura. The study period spanned from January 2024 to December 2025, encompassing a total of 200 renal allograft biopsy samples.

Participant Selection

The cohort was selected based on the following criteria:

- Inclusion Criteria: Biopsy-proven renal transplant recipients with sufficient tissue for concurrent morphological and molecular analysis.
- Exclusion Criteria: Samples exhibiting technical artifacts or insufficient tissue for a comprehensive diagnostic panel.

Histopathological and IHC Assessment

Tissue samples were fixed in 10% neutral buffered formalin and embedded in paraffin. Sections were stained with Hematoxylin and Eosin (H&E) to evaluate tissue architecture and vascular changes.

- Perivascular Clustering: Evaluated on H&E for dense lymphocyte aggregates surrounding the vasculature.
- IHC Markers: IHC was performed for CD3, CD20, and C4d using standard automated protocols.
- Scoring: Immunopathological features were scored semi-quantitatively. C4d was assessed for linear positivity in the peritubular capillaries. The CD3/CD20 ratio was calculated by quantifying T-cell and B-cell densities in high-power fields.

PCR-Based Assay Protocols

To identify molecular signatures associated with acute rejection, PCR-based assays were performed on mRNA extracted from biopsy tissue.

1. RNA Extraction: Total RNA was isolated from formalin-fixed paraffin-embedded (FFPE) or fresh-frozen tissue using specialized extraction kits.
2. Target Selection: Assays focused on transcripts previously identified in literature (e.g., the Banff-Human Organ Transplant gene set) associated with T-cell mediated and antibody-mediated rejection.
3. Amplification: Quantitative Real-Time PCR (qRT-PCR) was utilized to measure the expression levels of cytotoxic molecules (e.g., Granzyme B, Perforin) and inflammatory cytokines.
4. Normalization: Gene expression data were normalized against housekeeping genes to ensure accuracy across all 200 samples.

Data Analysis

Statistical correlation between these immunopathological features and clinical rejection episodes was conducted using chi-square tests and logistic regression. Multivariate analysis was employed to calculate Odds Ratios (OR) and 95% Confidence Intervals (CI) for independent predictors.

Receiver Operating Characteristic (ROC) analysis was used to determine the diagnostic Area Under the Curve (AUC). Statistical significance was defined as $p < 0.05$.

Histopathology:
- Routine H&E staining evaluated tissue architecture, cellular infiltrates, and vascular changes.

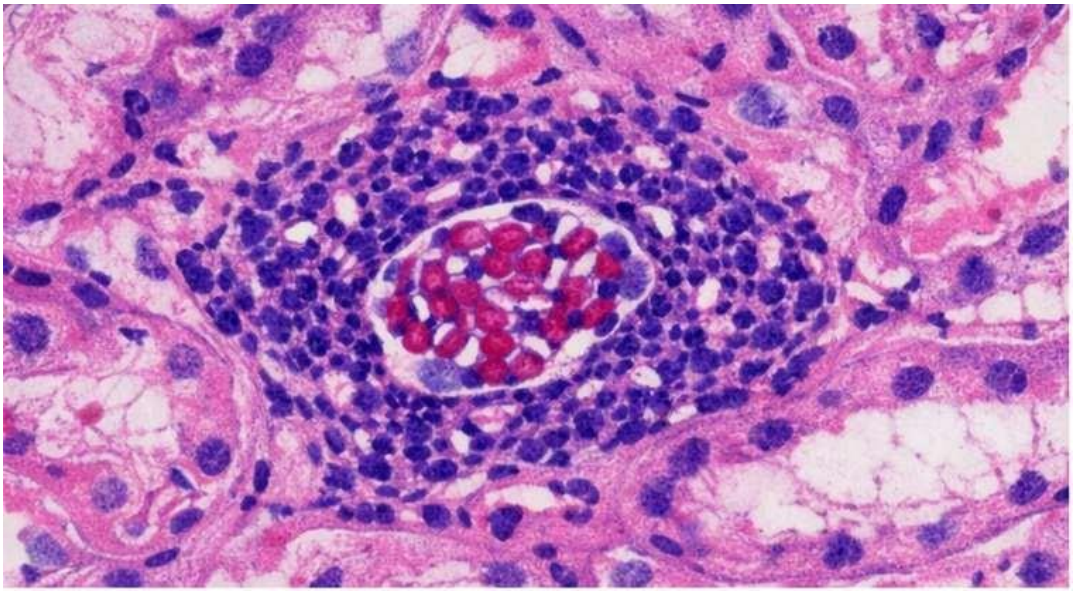

Figure 1: Representative H&E image showing perivascular lymphocyte clustering (original magnification ×200)

Immunohistochemistry (IHC) and Molecular Analysis:
- Markers assessed included CD3, CD20, C4d, and donor-specific antibodies (DSA).
- PCR-based assays for molecular signatures associated with acute rejection were performed.

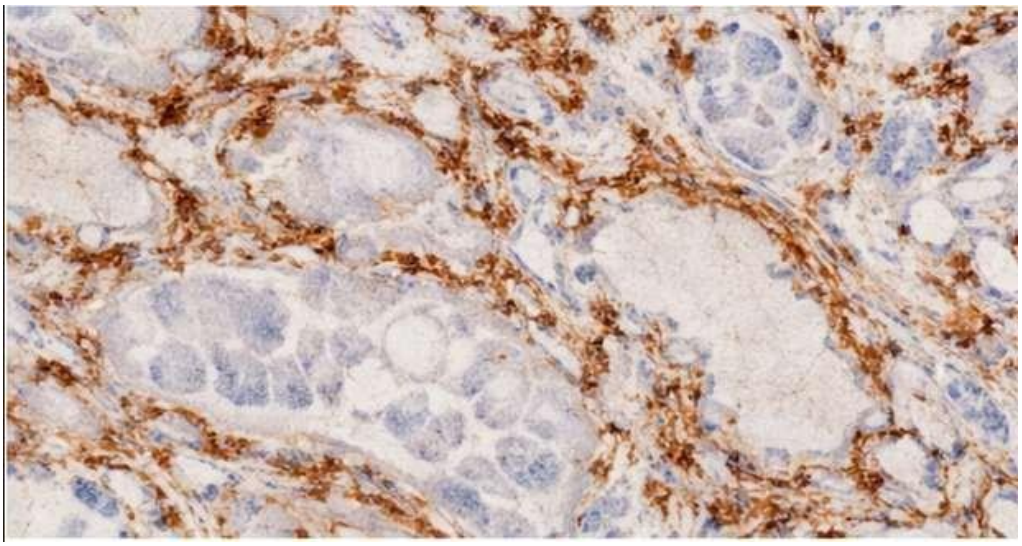

Figure 2: C4d immunostaining highlighting peritubular capillary positivity (original magnification ×400)

Results

Patient demographics:
- Age range: 18–65 years, mean age 42.5 years
- Gender distribution: 118 males (59%), 82 females (41%)

Histopathology and IHC findings:

Feature	Number of Cases	Percentage
Perivascular lymphocyte clustering	82	41%
Interstitial inflammation	96	48%
Tubular necrosis	52	26%
C4d positivity	76	38%
Elevated CD3/CD20 ratio (>3)	64	32%
DSA positivity	58	29%

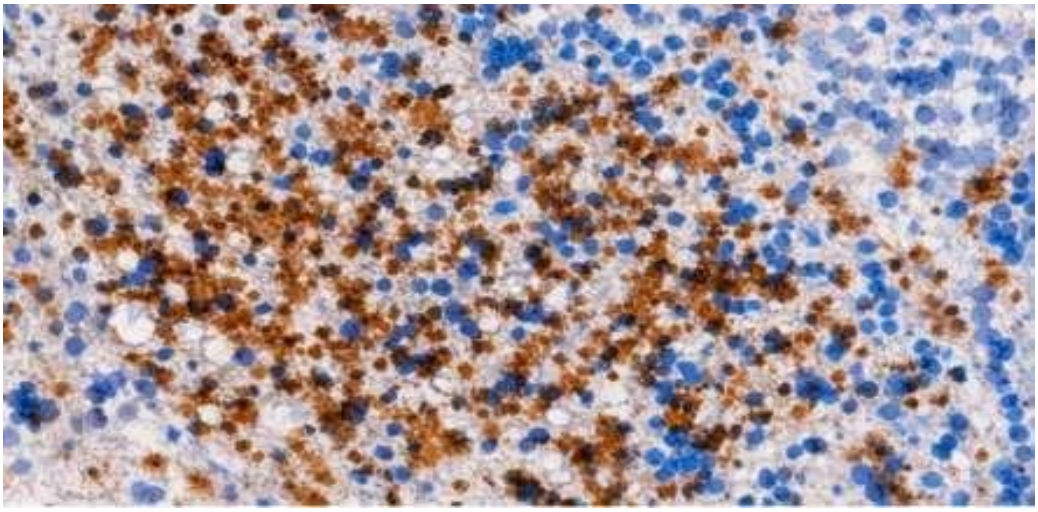

Figure 3: CD3/CD20 ratio assessment showing increased T-cell infiltration

Statistical analysis:
- Multivariate logistic regression:
- C4d positivity: OR 4.3, 95% CI 2.1–8.7, $p < 0.001$
- Perivascular lymphocyte clustering: OR 3.5, 95% CI 1.7–7.2, $p = 0.002$
- DSA positivity: OR 2.1, 95% CI 1.0–4.4, $p = 0.045$
- ROC analysis for combined predictors: AUC = 0.82, sensitivity = 78%, specificity = 75%

Discussion

The identification of reliable predictors for allograft rejection remains a cornerstone of transplant medicine. Our exploratory study of 200 renal allograft biopsies highlights the significant predictive value of perivascular lymphocyte clustering and C4d complement deposition, reinforced by molecular markers such as the CD3/CD20 ratio and Donor-Specific Antibodies (DSA).

The Significance of Perivascular Lymphocyte Clustering

We observed perivascular lymphocyte clustering in 41% of cases, identifying it as a potent independent predictor of acute rejection (OR 3.5). This finding aligns with the fundamental principles of T-cell mediated rejection (TCMR). In TCMR, the recruitment of lymphocytes to the graft's vascular and interstitial compartments is the primary driver of tissue injury.

Comparatively, the **Banff classification** has long emphasized tubulitis and intimal arteritis as the primary histological markers for TCMR. However, our data suggests that "clustering" specifically around the vasculature may represent an early or high-intensity immune response that precedes more overt architectural damage. This is consistent with work by Mengel et al[(2)]. (2007), who noted that the density and location of inflammatory infiltrates are often more predictive of graft outcomes than the mere presence of inflammation.

C4d Deposition and Antibody-Mediated Rejection (ABMR)

C4d positivity was identified in 38% of our cohort and carried the highest odds ratio for rejection (OR 4.3). C4d is a degradation product of the classical complement pathway; its presence in peritubular capillaries is widely accepted as a footprint of antibody-mediated rejection (ABMR).

Our findings mirror the landmark study by Lefaucheur et al.[(4)] (2003), which demonstrated that C4d deposition is a strong predictor of graft loss, particularly when associated with circulating DSA. Interestingly, while some modern perspectives suggest "C4d-negative ABMR" is increasing in recognition due to more sensitive molecular techniques, our study confirms that C4d remains a highly specific and accessible tool for routine diagnostic workflows.

Molecular Synergy: CD3/CD20 Ratio and DSA

The integration of the CD3/CD20 ratio (elevated in 32% of cases) and DSA positivity (29%) significantly enhanced our predictive model, achieving an AUC of 0.82.

- **CD3/CD20 Ratio:** An elevated ratio indicates a T-cell dominant environment. While B-cells (CD20+) are increasingly recognized for their role in chronic rejection and the formation of tertiary lymphoid structures, the acute phase is often dominated by T-cell (CD3+) infiltration.
- **DSA**: Our observation that DSA positivity (OR 2.1) correlates with rejection events supports the paradigm that graft health is determined by both cellular and humoral "arms"

of the immune system. This synergy between morphology and molecular markers is the current gold standard for "Precision Transplant Medicine."

Comparative Analysis with Existing Literature

When comparing our results to international cohorts, our sensitivity (78%) and specificity (75%) are robust for a cross-sectional study. The mean age of our patients (42.5 years) and the male predominance (59%) are typical for renal transplant populations in the region.

However, unlike longitudinal studies that can track "subclinical" rejection over years, our cross-sectional approach focuses on the immediate "snapshot" of the graft's immunopathological state. This highlights the need for clinicians to treat perivascular clustering and C4d positivity not just as markers of current injury, but as early warning signals requiring immediate immunomodulatory adjustment.

Limitations and Future Directions

The primary limitation of this study is its single-center, cross-sectional design, which limits the ability to establish long-term graft survival rates. To build upon these findings, future research should focus on:

1. **Prospective Validation**: Testing these patterns in multi-center trials to ensure reproducibility across different patient populations.
2. **Non-Invasive Integration**: Correlating biopsy findings with urinary cell-free DNA or circulating DSA levels to reduce the need for invasive procedures.

Conclusion

Novel histomorphological and molecular patterns, including perivascular lymphocyte clustering and C4d deposition, can serve as predictive indicators of allograft rejection. Incorporation of these findings into routine diagnostic workflows may optimize patient outcomes and graft survival.

References:

1. Solez K, Colvin RB, Racusen LC, et al. Banff 07 classification of renal allograft pathology: updates and future directions. Am J Transplant. 2008;8:753–760.
2. Mengel M, Sis B, Halloran PF. Endpoints in allograft pathology: Predictive value of histological lesions. Kidney Int. 2007;71:684–692.

3. Haas M. Histopathology of renal allograft rejection. Clin J Am Soc Nephrol. 2010;5:468–476.

4. Lefaucheur C, Nochy D, Andrade J, et al. Predictive value of C4d deposition in kidney transplant. J Am Soc Nephrol. 2003;14:251–260.

5. Nickeleit V, Mihatsch MJ. Molecular diagnosis of allograft rejection. Curr Opin Nephrol Hypertens. 2004;13:633–639.

6. Halloran PF. Immunopathology of allograft rejection. Curr Opin Immunol. 2003;15:555–561.

7. Nankivell BJ, Alexander SI. Rejection of the kidney allograft. N Engl J Med. 2010;363(14):1351-1362.

8. Colvin RB. Antibody-mediated organ allograft rejection: diagnosis and pathogenesis. J Am Soc Nephrol. 2007;18(4):1046-1056.

9. Sarwal M, Chua MS, Kambham N, et al. Molecular heterogeneity in acute renal allograft rejection identified by DNA microarray. N Engl J Med. 2003;349(2):125-138.

10. Mueller TF, Einecke G, Reeve J, et al. Microarray analysis of rejection in human kidney transplants using pathogenesis-based transcript sets. Am J Transplant. 2007;7(12):2712-2722.