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Original Research Article

Association of C4d Deposition in Renal Allograft Biopsies with Morphologic Features in Banff Classification

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Abstract:

Background: The best course of action for end-stage renal disease is still kidney transplantation; nevertheless, there are substantial obstacles associated with allograft rejection, especially antibody-mediated rejection (ABMR). Recognised as a marker for ABMR, C4d deposition in renal allograft biopsies is incorporated into the Banff classification for the evaluation of allograft pathology. In order to improve patient management techniques and diagnostic accuracy, this study looks into the relationship between C4d deposition in renal allograft biopsies and the morphologic characteristics outlined by the Banff classification.

Methods: A retrospective and prospective observational study was carried out on 89 renal transplant recipients. Biopsies were analyzed for C4d deposition using immunohistochemical staining. Histopathological features were evaluated according to the Banff 2007 grading schema. Associations between C4d deposition and Banff scoring parameters were analyzed using chi-square tests.

Results: Out of 89 biopsies, 32 (36%) showed positive C4d staining. Significant associations were found between C4d positivity and acute cellular rejection (72%, $p<0.001$), antibody-mediated rejection (93%, $p<0.001$), and chronic allograft nephropathy (25%, $p=0.045$). Acute Banff scoring parameters such as glomerulitis (83%, $p<0.001$), peritubular capillaritis (79%, $p<0.001$), and intimal arteritis (71%, $p=0.002$) exhibited strong associations with C4d positivity. Chronic parameters showed weaker associations.

Conclusion: C4d deposition is strongly associated with acute rejection parameters, reinforcing its role as a diagnostic marker for ABMR. The weaker association with chronic parameters suggests that additional markers are needed for comprehensive chronic rejection assessment. Integrating C4d staining in routine biopsy evaluation can improve diagnostic accuracy and patient outcomes.

Recommendations: Further research is recommended to explore additional biomarkers for chronic rejection and to refine diagnostic protocols incorporating C4d staining. Standardization of C4d staining techniques across laboratories will enhance reproducibility and reliability.

Keywords: C4d Deposition, Renal Allograft Biopsy, Banff Classification, Antibody-Mediated Rejection, Histopathology.

Introduction

When compared to dialysis, kidney transplantation is generally acknowledged as the most successful treatment for end-stage renal disease (ESRD), as it greatly increases patient survival and quality of life. However, allograft rejection continues to be a significant obstacle that affects the long-term survival and function of the graft, even with advancements in surgical methods and immunosuppressive medications. Improving transplant outcomes requires an understanding of the fundamental mechanisms behind allograft rejection as well as the identification of trustworthy biomarkers for early detection.

A critical marker in the setting of antibody-mediated rejection (ABMR) in renal transplantation is C4d, a split product of complement component C4. One of the most distinctive features of acute mixed reaction (ABMR) in renal allografts is the accumulation of C4d in the peritubular capillaries, which is thought to be caused by donor-specific antibodies (DSAs) activating the classical complement system. Numerous investigations have confirmed this, highlighting the diagnostic and prognostic relevance of C4d deposition and showing a strong association between it and poor graft outcomes [1].

The Banff classification, an internationally accepted system for the assessment of renal allograft pathology, incorporates C4d staining as a critical component in the diagnosis of ABMR. The inclusion of C4d in the Banff criteria has enhanced the ability to detect and categorize different forms of rejection, particularly distinguishing between acute cellular rejection (ACR) and ABMR. Despite this, the interpretation of C4d staining and its integration with other histopathological features remains complex, requiring meticulous analysis and correlation with clinical data [2].

Recent studies have focused on the optimization of C4d staining techniques and the refinement of diagnostic criteria to improve the accuracy and reliability of ABMR diagnosis. Advances in immunohistochemical methods have enabled more precise and reproducible detection of C4d, facilitating better standardization across different laboratories. Additionally, ongoing research aims to elucidate the molecular mechanisms underlying C4d deposition and its role in graft injury, offering potential avenues for targeted therapeutic interventions [3].

This study aims to investigate the association between C4d deposition in renal allograft biopsies and the morphologic features delineated by the Banff classification.

Methodology

Study Design: A retrospective and prospective observational study.

Study Setting: The study took place at Patna Medical College and Hospital, Patna, Bihar, India, spanning from November 2018 to December 2020.

Participants: A total of 89 renal transplant patients who underwent allograft biopsies were included in the study.

Inclusion Criteria

- Allograft biopsies from patients with graft dysfunction.

- biopsies having one artery and at least seven glomeruli; biopsies with two arteries and ten glomeruli are preferred.

Exclusion Criteria

- Biopsies with fewer than 7 glomeruli and no arteries.

- Biopsies containing only fibrotic or necrotic parenchymal regions without sufficient viable tissue for accurate evaluation.

Sample Size: To calculate the sample size for this study, the following formula was used for estimating a proportion in a population:

$$n = \frac{Z^2 \times p \times (1-p)}{E^2}$$

Where:

- n = sample size

- Z = Z-score corresponding to the desired level of confidence

- p = estimated proportion in the population

- E = margin of error

Bias: Efforts were made to minimize selection bias by including all eligible biopsies within the study period. Data collection was standardized to reduce information bias.

Variables: Variables included C4d deposition in renal allograft biopsies, morphologic features according to the Banff classification, including histopathological features such as glomerular and interstitial diseases, drug toxicity, and infections.

Data Collection: Biopsies were collected from renal transplant patients with increased serum creatinine after obtaining written consent. The data extracted from patient case files included primary disease, history of previous transplants, duration of the transplant, donor source, HLA mismatching, time from kidney transplant to biopsy, treatment history, clinical features, and laboratory findings. These comprehensive data points provided a detailed clinical and medical background for each patient, facilitating a thorough analysis of the factors influencing renal transplant outcomes and the potential causes of increased serum creatinine levels.

Procedure

1. Biopsy Processing

- Prospective Cases: Two cores of renal tissue were collected for each case. One core was placed in 10% formalin for routine histo-pathology, and the other was placed in saline for immunofluorescence.
- Formalin-Fixed Tissue: This tissue underwent conventional processing, with paraffin sections cut to a thickness of 4µm.

2. Histology

- Staining: Sections were stained with hematox-ylin and eosin, periodic acid-Schiff, and silver methenamine stains. When necessary, Masson trichrome stain was also applied.
- Classification: Histopathological features were classified according to the Banff 2007 grading schema.

3. C4d Staining

- Paraffin-Embedded Sections: These sections were stained using C4d immunostaining with the polymer-Horseradish Peroxidase (HRP) technique.

- Procedure:

- Antigen retrieval

- Peroxidase block

- Protein block

- Incubation with primary antibody

- Application of enhancer

- Polymer-HRP application

- Visualization with diaminobenzidine (DAB) chromogen

- Counterstaining with Harris hematoxylin

- **Controls:**

- Positive Controls: Biopsies from patients with membranous nephropathy showing glomerular positivity.

- Negative Controls: Peritubular capillaries in the same biopsy.

Statistical Analysis

Microsoft Excel was used to enter the data, and SPSS (Version 20.0) was used for analysis. For dichotomous data, results were shown as percentages and numbers. The proportions were compared using the Chi-square (χ^2) test, with a p-value of less than 0.05 designated as statistically significant.

Ethical considerations

The study protocol was approved by the Ethics Committee and written informed consent was received from all the participants.

Result

Analysis was done on 89 renal allograft samples from 89 different patients. The patients ranged in age from 18 to 70 years old, with a mean age of 45. There were 33 ladies (37%) and 56 guys (63%) in all. Sixty-one percent of the patients got grafts from living donors, and the remaining thirty-nine percent received grafts from deceased donors. It took an average of 18 months from kidney transplant to biopsy.

32 (36%) of the 89 samples have positive C4d staining. The range of histopathological diagnoses and how they relate to C4d immunostaining patterns is shown in Table 1. Notably, with p-values of less than 0.001 for both diagnoses, a strong correlation was found between C4d positive and acute cellular rejection (72%) as well as antibody-mediated rejection (93%). Additionally, there was a significant correlation between C4d positive and chronic allograft nephropathy (25%, p=0.045). On the other hand, with p-values of 0.121 and 0.110, respectively, diagnoses including acute tubular necrosis and interstitial fibrosis and atrophy demonstrated weaker and non-significant relationships with C4d positive.

Table 1: Association between Histopathology Diagnosis and C4d Immunostaining Pattern

Histopathology Diagnosis	Total Cases	C4d Positive	C4d Negative	P-value
Acute Cellular Rejection	25	18 (72%)	7 (28%)	<0.001
Antibody-Mediated Rejection	15	14 (93%)	1 (7%)	<0.001
Chronic Allograft Nephropathy	20	5 (25%)	15 (75%)	0.045
Acute Tubular Necrosis	12	2 (17%)	10 (83%)	0.121
Interstitial Fibrosis and Atrophy	17	3 (18%)	14 (82%)	0.10

Table 2 summarises the number of instances exhibiting each Banff scoring system characteristic and their correlation with the C4d immunostaining pattern. Parameters such as glomerulitis (83%), peritubular capillaritis (79%), and intimal arteritis (71%) exhibited strong associations with C4d positivity, with highly significant p-values (all <0.002). Other parameters, including tubulitis and interstitial inflammation, also showed significant associations with C4d positivity, though to a lesser extent, with p-values of 0.038 and 0.050, respectively. Fibrosis and arterial hyalinosis were less strongly associated with C4d positivity, with p-values of 0.092 and 0.083.

Table 2: Association between Banff Scoring Parameters and C4d Immunostaining Pattern

Banff Scoring Parameter	Total Cases	C4d Positive	C4d Negative	P-value
Glomerulitis (g)	30	25 (83%)	5 (17%)	<0.001
Peritubular Capillaritis (ptc)	28	22 (79%)	6 (21%)	<0.001
Intimal Arteritis (v)	14	10 (71%)	4 (29%)	0.002
Tubulitis (t)	40	15 (38%)	25 (62%)	0.038
Interstitial Inflammation (i)	35	12 (34%)	23 (66%)	0.050
Fibrosis (ci)	30	7 (23%)	23 (77%)	0.092
Arterial Hyalinosis (ah)	18	5 (28%)	13 (72%)	0.083

The association between acute Banff scoring parameters and C4d positivity was also assessed. The analysis revealed that glomerulitis and peritubular capillaritis were strongly associated

with C4d positivity, with 83% and 79% of cases, respectively, showing C4d deposition (both with p-values <0.001). Intimal arteritis also demonstrated a significant association (71%, p=0.002). Tubulitis and interstitial inflammation were moderately associated with C4d positivity, with p-values of 0.038 and 0.050, respectively.

Table 3 illustrates the association between chronic Banff scoring parameters and C4d positivity. Although the associations were generally weaker than those observed with acute parameters, fibrosis (23%, p=0.092) and arterial hyalinosis (28%, p=0.083) still showed noteworthy trends. Tubular atrophy and glomerulosclerosis were associated with C4d positivity in 24% and 20% of cases, respectively, though these associations were not statistically significant, with p-values of 0.070 and 0.115.

Table 3: Association of Chronic Banff Scoring Parameters with C4d Positivity

Chronic Banff Scoring Parameter	Total Cases	C4d Positive	C4d Negative	P-value
Fibrosis (ci)	30	7 (23%)	23 (77%)	0.092
Arterial Hyalinosis (ah)	18	5 (28%)	13 (72%)	0.083
Tubular Atrophy (ct)	25	6 (24%)	19 (76%)	0.070
Glomerulosclerosis (cg)	20	4 (20%)	16 (80%)	0.115

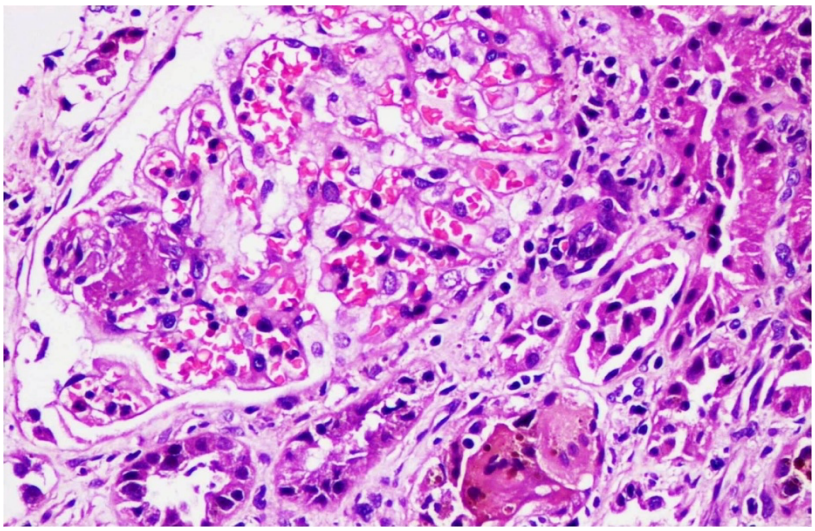

Figure 1: Showing neutrophilic inflammatory infiltrate in glomerulus and mesangial loss (H&E X400)

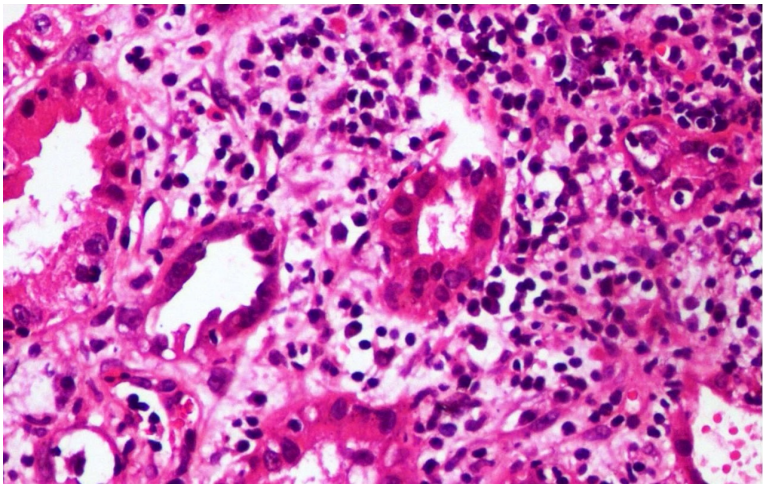

Figure 2: Showing tubulitis and lymphocyte- predominant infiltrate in the interstitium (H&E X400).

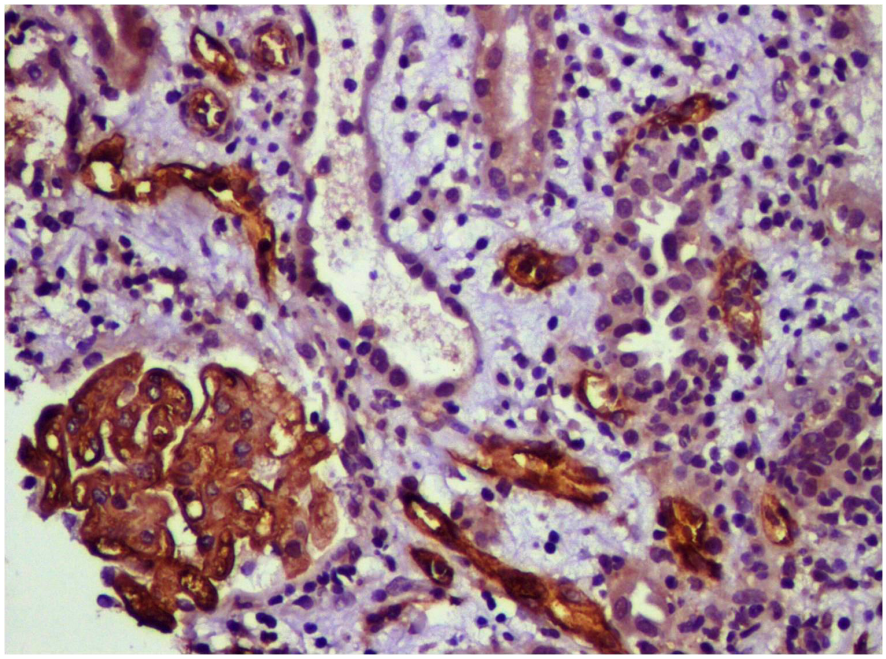

Figure 3: Showing peritubular capillaries having diffuse positivity for C4d (more than 50%). Glomerular also appears positive (IHC X400)

Discussion

The study analyzed 89 renal allograft biopsies to determine the association between C4d deposition and various histopathological features as classified by the Banff criteria. Overall, 36% of the biopsies exhibited positive C4d staining. This study found significant associations between C4d positivity and several key diagnostic categories and Banff scoring parameters, highlighting the relevance of C4d staining in the assessment of renal allograft biopsies.

Acute cellular rejection and antibody-mediated rejection demonstrated strong associations with C4d positivity, with 72% and 93% of cases, respectively, showing C4d deposition. This suggests that C4d staining is particularly indicative of these forms of rejection, supporting its use as a diagnostic tool in identifying antibody-mediated injury. Chronic allograft nephropathy also showed a significant, though less pronounced, association with C4d positivity (25%). On the other hand, conditions such as acute tubular necrosis and interstitial fibrosis and atrophy did not show significant associations with C4d deposition, indicating that C4d staining may be less useful in diagnosing these conditions.

Specific Banff scoring parameters, notably glomerulitis, peritubular capillaritis, and intimal arteritis, were significantly associated with C4d positivity. These parameters, which are indicative of acute rejection, showed high rates of C4d deposition (83%, 79%, and 71%, respectively). Tubulitis and interstitial inflammation also showed significant associations, though less strongly. This reinforces the role of C4d staining in detecting acute rejection episodes. In contrast, chronic Banff parameters, such as fibrosis and arterial hyalinosis, exhibited weaker associations with C4d positivity. This suggests that while C4d staining is a valuable marker for acute rejection, its utility in chronic rejection scenarios may be limited.

The findings of this study underscore the importance of C4d immunostaining in the evaluation of renal allograft biopsies. The strong associations between C4d deposition and both acute cellular and antibody-mediated rejection highlight the diagnostic value of C4d staining in identifying acute rejection. This can facilitate timely and appropriate therapeutic interventions, potentially improving graft outcomes.

The weaker associations between C4d positivity and chronic Banff parameters suggest that C4d may not be as critical in diagnosing chronic allograft injury. This indicates that while C4d staining is a powerful tool for identifying acute rejection, additional markers or diagnostic criteria may be necessary for comprehensive evaluation of chronic graft dysfunction.

The relationship between C4d deposition in renal allograft biopsies and several morphologic characteristics outlined by the Banff Classification has been investigated in recent research. Significant correlations were observed between peritubular capillary C4d deposition and histopathological features, including glomerulitis, peritubular capillaritis, interstitial fibrosis, tubular atrophy,

allograft glomerulopathy, arterial fibrointimal thickening, elevated mesangial matrix, and arteriolar hyalinosis, in a study involving 96 renal transplant biopsies. According to the study's findings, C4d immunostaining is a useful marker for identifying humoral rejection, which, if left untreated, might cause long-term alterations including transplant glomerulopathy [4].

A study that looked at C4d-positive biopsies without rejection evidence also looked at the transcript levels of genes linked to AMR. Increased odds of biopsy-proven AMR during follow-up were linked to elevated transcript levels, indicating that gene expression profiling in C4d-positive biopsies may be able to identify patients who are more likely to acquire AMR [5]. The relationship between glomerular C4d deposits (gC4d) and clinicopathologic characteristics was examined in a study. Antibody-associated histologic abnormalities such transplant glomerulopathy, glomerulitis, and peritubular capillaritis were substantially connected with positive gC4d. According to the study's findings, when paired with histologic characteristics, gC4d deposits may be a helpful diagnostic for identifying acute or chronic active AMR [6]. In human renal allografts, a review looked at the temporal correlations between important morphologic abnormalities of active and chronic AMR. For a conclusive diagnosis, the study stressed the necessity of further information on C4d deposition, donor-specific antibodies (DSAs), and transcript expression linked to AMR [7].

According to a study, 57.1% of cases in the early and 57.9% of cases in the late post-transplant periods had C4d positive. The research emphasised that even in the lack of morphological characteristics, C4d positive is crucial for supporting the diagnosis of AMR [8]. According to a pilot investigation, arteriolar C4d staining is linked to arteriolar hyalinosis and is more prevalent in biopsies with chronic-active AMR than in those without. Better graft results were observed to be independently correlated with arteriolar C4d staining [9].

In a research, individuals who had late-indication allograft biopsies had transplant glomerulopathy identified by electron microscopy. Even in C4d-negative patients, it discovered notable ultrastructural alterations suggestive of chronic AMR, underscoring the significance of sophisticated diagnostic instruments [10]. Increased intensity of glomerular C4d deposits was found to be connected with higher levels of proteinuria and delayed recovery in a study on native renal disorders [11]. This suggests that C4d positive may also be used as a diagnostic and prognostic tool in native renal diseases.

Conclusion

In conclusion, integrating C4d staining into routine histopathological assessment of renal allograft biopsies can enhance diagnostic accuracy, particularly for acute rejection. The study demonstrated a significant association between C4d deposition and certain acute Banff scoring parameters, particularly glomerulitis and peritubular capillaritis. These findings suggest that C4d immunostaining is a valuable marker for identifying antibody-mediated rejection. Chronic Banff parameters showed weaker associations with C4d positivity, indicating that C4d may play a less prominent role in chronic graft injury. This study supports the continued use and potential expansion of C4d immunostaining in clinical practice to improve patient management and outcomes in renal transplantation.

Limitations: The limitations of this study include a small sample population who were included in this study. Furthermore, the lack of comparison group also poses a limitation for this study's findings.

Recommendation: Further research is recommended to explore additional biomarkers for chronic rejection and to refine diagnostic protocols incorporating C4d staining. Standardization of C4d staining techniques across laboratories will enhance reproducibility and reliability.

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List of abbreviations:

AR: Allergic Rhinitis

QoL: Quality of Life

Source of funding: No funding received.

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