Renal allograft biopsies at Groote Schuur Hospital: A histopathologic descriptive study with molecular insights

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Declaration

I, **JARRYD LUNN,** student number **LNNJAR001**, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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A full Turnitin report is attached to this submission with form D19 (see ‘appendix 9.7).

Signed by candidate

Jarryd Lunn
15/04/2025

Project acknowledgements

I give thanks to my supervisors, department, fellow registrars and consultants of my division for their input and guidance on this project. I also give thanks to Dr Joanne Lu, who greatly contributed to data collection.

Dedication

I dedicate this thesis to:

- LL, WL, SL, OBL, NS, AAB, BG, CA, CLRS, ED, JMC, MH, PG, AS, RW, WT, and CD for their presence, kindness and support;
- 소녀시대 & 트와이스;
- Every soul I was kahu to who taught me about love and simple joys;
- Al farabutto che scrisse 'la vita e bella baco' su un muro di Corso Italia a Milano; questa frase mi ha ricordato di vivere, amare e trovare la mia felicità;

Infine, lo dedico a Dio, la cui Presenza ancora sento e riconosco silenziosamente.

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Manuscript title

Streamlining renal rejection diagnosis in a resource-limited setting: The impact of Banff 2022 criteria on a South African cohort, with assessment of C4d as a predictor of donor specific antibodies

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Brendon Price:	Conceptualization, project administration, editing, supervision

Conflict of interest

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

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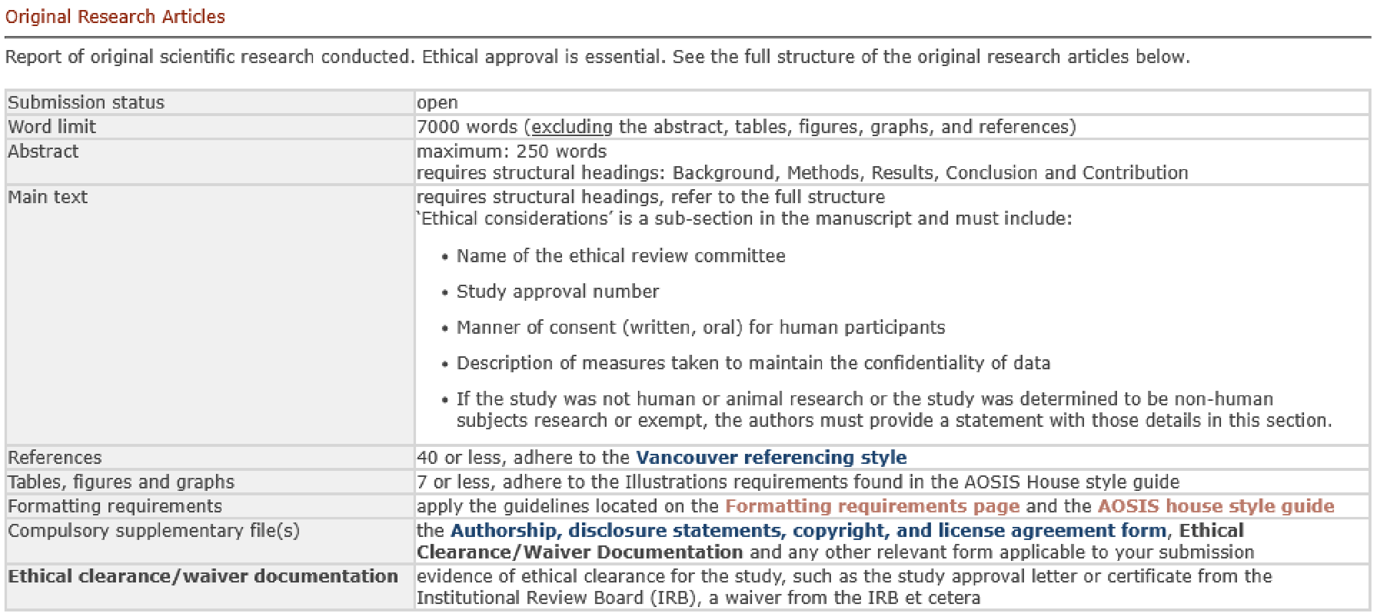
Data availability statement

Raw data were generated at Groote Schuur Hospital. Derived data supporting the findings of this study are available from the corresponding author, [BP], on reasonable request.
Data capture tool available at link: Data capture instrument

Instructions for authors (summary)

The below summary is taken from the following link: Journal of the CMSA - submission guidelines



Original Research Articles

Report of original scientific research conducted. Ethical approval is essential. See the full structure of the original research articles below.

Submission status	open
Word limit	7000 words (excluding the abstract, tables, figures, graphs, and references)
Abstract	maximum: 250 words requires structural headings: Background, Methods, Results, Conclusion and Contribution
Main text	requires structural headings, refer to the full structure 'Ethical considerations' is a sub-section in the manuscript and must include: • Name of the ethical review committee • Study approval number • Manner of consent (written, oral) for human participants • Description of measures taken to maintain the confidentiality of data • If the study was not human or animal research or the study was determined to be non-human subjects research or exempt, the authors must provide a statement with those details in this section.
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Ethical clearance/waiver documentation	evidence of ethical clearance for the study, such as the study approval letter or certificate from the Institutional Review Board (IRB), a waiver from the IRB et cetera

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Abbreviations

Banff Lesion Score abbreviations

i	Interstitial inflammation
t	Tubulitis
v	Intimal arteritis
g	Glomerulitis
ptc	Peritubular capiliritis
C4d	Complement degradation product C4d
ci	Interstitial fibrosis
ct	Tubular atrophy
cv	Vascular fibrous intimal thickening
cg	Transplant glomerulopathy (GBM double contours)
mm	Mesangial matrix expansion
ah	Arteriolar hyalinosis
aah	Hyaline arteriolar thickening
ti	Total inflammation
i-IFTA	Inflammation in the area of IFTA
t-IFTA	Tubulitis in areas of interstitial fibrosis
pvl	Polyomavirus load

Other

AUC	Area under the curve
CI	Confidence interval
ESKD	End stage kidney disease
HIV	Human Immunodeficiency Virus
IQR	Interquartile range
NHLS	National Health Laboratory Service

Rejection-related abbreviations

ABMR	Antibody-mediated rejection (pre-2017)
AMR	Antibody-mediated rejection
‘Borderline’	Suspicious (borderline) for acute TCMR
DSA	Donor specific antibodies
MVI	Microvascular inflammation
TCMR	T cell-mediated rejection

Non-rejection related pathology

ATI	Acute tubular injury
BK	BK-polyomavirus infection
CIT	Calcineurin inhibitor toxicity
DIN	Drug induced interstitial nephritis
DNG	De novo glomerulopathy
GBM	Glomerular basement membrane changes
OU	Obstruction
PN	Pyelonephritis
RD	Recurrence of (primary) disease
SC	Subcapsular injury

1 Abstract

Background: Kidney transplantation is the definitive treatment for end-stage kidney disease. Immune-mediated rejection remains a barrier to success. It is diagnosed through the Banff classification, which incorporates histopathology and biomarkers (C4d/donor specific antibodies (DSA)). In resource-limited settings, DSA testing can be challenging, necessitating reliable alternatives.

Aim: This study evaluated: (1) rejection patterns at our hospital; (2) the impact of the Banff 2022 criteria with a computer-assisted tool; and (3) the utility of C4d as a predictor of DSA status.

Methods: We analysed 197 for-cause historic biopsy reports between 2015-2022 for details of rejection- and non-rejection pathologies, Banff lesion scores and DSA status. A computer-based tool was used on historic data to re-calculate Banff 2022 classification diagnoses, which were compared to historic diagnoses. Logistic regression assessed C4d as a predictor of DSA.

Results: The cohort showed a male predominance (59.3%). Sixty-three percent of cases showed non-rejection pathology, with acute tubular injury and pyelonephritis being the most frequent. TCMR was the most common form of rejection (17.3%), with AMR being the least common (7.6%). The computer-based tool demonstrated agreement of 92.4% for AMR/TCMR and 84.6% of borderline TCMR, but was confounded by non-rejection pathologies. C4d predicted DSA-positivity with 95% specificity but only 29.5% sensitivity.

Conclusion: The Banff 2022 criteria were additive in rejection diagnosis, with a computer-based tool acting as a guide but not a pure diagnostic tool. The high specificity of C4d makes it valuable where DSA testing is limited.

Contribution: This study validates the role of the Banff 2022 in our setting, aided by a computer-based tool that aims to decrease logical- and transcription errors when using the complex Banff classification. It also demonstrates C4d's role as a practical DSA proxy, offering actionable solutions in resource-limited settings.

(281 words)

Key words: Renal transplantation, Banff 2022 classification, renal rejection prevalence, rejection biomarkers, digital pathology algorithm, C4d-DSA prediction, Antibody mediated rejection, T-cell mediated rejection

2 Background

Chronic kidney disease, affecting over five million South Africans(1), often progresses to end stage kidney disease (ESKD) where patients become dependent on renal replacement therapy (dialysis) or renal transplantation for survival(2-4). The definitive treatment for ESKD is renal transplantation which improves life expectancy and quality of life when compared to dialysis(5), and is thus a critical service in modern nephrology.

Although transplant success rates have significantly improved over the last seven decades, success is often hindered by immune-mediated rejection(6). An episode of acute rejection is a significant risk factor for graft loss, demonstrated in western populations(7, 8). Given this risk, urgent and accurate histopathologic diagnosis of rejection is essential to guide clinical decision-making.

The need for diagnostic guidelines for renal rejection gave rise to the Banff consensus meetings on allograft pathology, which started in 1991(9). These have been the cornerstone of rationalized, measurable histopathologic diagnoses in renal allograft pathology and rejection. Biennial iterations of the Banff criteria have provided an evolving framework for the histopathological diagnosis of renal allograft-associated pathology(9-11), which aid in clinical decision making.

The framework consists of semi-quantitative ‘Banff lesion scores’ based on the visualized histopathology of various compartments of renal tissue (glomeruli, tubules, interstitium and vasculature), enabling a standardized and comparable diagnostic approach(9). Initially based on expert opinion, the system is now heavily evidence-based, with large cohort studies and meta-analyses guiding changes(9). While a detailed discussion of these guidelines is beyond this article’s scope, they are eloquently explained by the Banff Foundation’s recent updates (10, 12).

The Banff consensus guidelines are updated every two years; an excellent summary of the year-on-year changes is given in a review article by Loupy *et al*(9). These iterations aim to improve the diagnostic sensitivity and specificity of guidelines, given the increasing dataset from which diagnostic criteria are decided. Each iteration hence has the potential to more accurately characterize and identify cases of renal rejection(9, 10).

Rejection is classified broadly as ‘T-cell mediated rejection’ (TCMR) and ‘antibody mediated rejection’ (AMR), both of which require urgent but different clinical management to ensure graft survival(13, 14).

TCMR is a cellular immune response to allograft human leukocyte antigen (HLA) molecules, which induces a directed cellular response against the transplanted kidney, manifesting histologically as interstitial inflammation, tubulitis, interstitial fibrosis and tubular atrophy(10, 12, 15). TCMR is categorized into acute and chronic active forms. In cases where there are insufficient criteria for a diagnosis of TCMR, and no intimal arteritis is present, a diagnosis of suspicious (borderline) for acute TCMR can be utilized(12). Ancillary studies are generally not needed in the diagnosis of TCMR.

TCMR remains the commonest form of rejection, having an overall prevalence of 11–30%, and 5–15% within the first year in international cohorts(7, 16). Importantly, evidence suggests that a single episode of TCMR places a recipient at risk of a future episode: nearly two thirds of those with an episode of TCMR had evidence of TCMR in future biopsies in a recent publication by Rampersad *et al*(7), which highlighted the importance of identification of TCMR. A single-centre South African study showed a higher risk of TCMR, with a 37% risk of TCMR within the first year, possibly reflecting resource limitations within our setting(4).

AMR is an antibody-mediated attack of the transplant endothelium, specifically of the glomerular and tubular capillaries (so-called microvascular inflammation, MVI), and to a lesser extent medium- and large arteries(8, 17, 18). AMR was first recognized by Banff in 1997, with the first criteria for AMR defined in 2001(9). It is less studied, and seemingly less common, with overall prevalence from 1–21% in studies, most around 3–12%(19). Nevertheless, it is an important risk factor for graft loss(8). In our setting, Davidson *et al* showed an increased incidence of 34% within the first month, and 36% between 1 and 5 years, possibly reflecting the effects of resource limitation in our setting(4).

The alloimmune antibody response to the transplanted kidney is mediated by anti-HLA class I/II donor specific antibodies (DSA), targeting antigens on the donor organ endothelium(17, 20, 21). DSA are thus a strong predictor of AMR, and both pre-formed (pre-transplant) and *de novo* DSA are shown to play a role in the spectrum of AMR(21). The Banff 2022 guidelines continue to recommend DSA assessment for a diagnosis of AMR (with some nuances, discussed below)(22). Detection of DSA around the time of biopsy is critical.

The laboratory measurement of DSA can be complex, with variability in available assays, difficulties with assay interferences, as well as the lack of standardized interpretation guidelines, discussed in depth by Haarberg *et al*(23). Furthermore, as is done in our setting, analysis is often batched due to the cost of the assay, delaying results and thus possibly delaying directed treatment of suspected rejection. Equipment breakdowns run the risk of halting DSA assessment, with obvious consequences for diagnosis of AMR.

The Banff consensus guidelines on renal rejection have long since recognised the role of DSA in rejection. Since 2001(24), Banff has also recognized the important role of the complement degradation product C4d as a histologic hallmark of AMR(22, 25-27). C4d is formed as part of the classical complement pathway, activated by DSA during episodes of AMR. A by-product without fully understood function, C4d bonds covalently to endothelium, making it a robust, stable histologic marker, and is thus a pragmatic surrogate marker for DSA-mediated endothelial injury(28).

C4d is easily assessed on biopsy tissue, either through the more sensitive immunofluorescent techniques, or through standard immunohistochemistry(12); at our laboratory at Groote Schuur Hospital, immunohistochemistry is used. Given this relative ease of assessment, C4d status can be rendered at the same time as the histologic assessment of Banff lesion scores, significantly faster than serum assessment of DSA.

Thus, given the underlying biology of C4d making it a surrogate for DSA status, it can aid in identifying patients at high risk for AMR before DSA results are available, and potentially decrease time to definitive clinical treatment. This has been demonstrated by several research groups(28-30), mostly in high-resource settings. To this end, the current Banff 2022 recognizes C4d in this light: C4d status in certain AMR categories can be substituted for DSA in providing molecular evidence of antibody mediated rejection(12, 22), which may expedite treatment in a resource-limited setting.

The evidence described by Davidson *et al*(4) from our hospital (spanning 2010–2015), which showed increased prevalence of both TCMR and AMR compared to international cohorts, highlights the importance of diagnosing these lesions accurately and timeously in our setting. While the HIV-positive to HIV-positive cohort has been recently studied(31), HIV-negative patients have not yet been studied for comparison since 2015 at our hospital.

Aims of this study

In this study we aimed to investigate the following:

(1) Characterize the landscape of renal allograft rejection and non-rejection pathologies within our hospital from 2015-2022: We described the renal transplant population based on historic biopsy reports at Groote Schuur Hospital from 2015–2022, and compare it to locally available data;

(2) Evaluate the diagnostic impact of Banff 2022 on historic diagnoses, using a computer-based tool: We applied the Banff 2022 guidelines to historic pathology reports to assess agreement between the new criteria and historic data, using a simple, automated computer-based tool to identify cases;

(3) Assess C4d as a surrogate marker for DSA at our hospital: In our setting where DSA are not always readily available, we assessed the performance of C4d immunohistochemistry as a predictor of DSA positivity.

3 Methods

3.1 Ethical approval

This study was approved by the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee (FHS-HREC 130/2022).

3.2 Study design, setting and population

This retrospective cohort study assessed renal transplant biopsy reports from patients at Groote Schuur Hospital for suspected rejection between September 2015 – December 2022. Patients were identified via Department of Nephrology biopsy records, and the National Health Laboratory Service (NHLS) TrakCare platform using matching SNOMED Clinical Terms(32). Any biopsied patient was included, except for HIV-positive patients who were excluded given the recent eloquent assessment of this cohort by Wearne *et al*(31). Our hospital does not perform protocol biopsies, and so all biopsies were for-cause. No slide review or human re-interpretation of the reported data was performed, in keeping with methods in similar studies in our setting(4, 31).

3.3 Data collection

Collected data from available histologic reports included demographics, all recorded Banff lesion scores, non-rejection pathology, and the final histologic diagnosis (referred to as the “reported diagnosis": recorded as positive, suspicious, or negative for rejection). At our laboratory, C4d immunohistochemistry is used (and not C4d immunofluorescence), and C4d scores were recorded.

Available serologic data (HIV status and DSA) were extracted for all patients. DSA testing done peri-biopsy (generally within 1 week before/after biopsy) was assessed to determine DSA status at the time of biopsy. DSA status was determined based on the reported DSA interpretation by NHLS Tissue Immunology laboratory at Groote Schuur Hospital. Briefly, this laboratory has access to confidential donor HLA profiles and compare these to the recipient serum profiles to detect donor-specific antibodies against the donor organ. Reports that had no explicit interpretation were excluded, as were DSA that were not done peri-biopsy, as defined above.

3.4 Data analysis

De-identified data were collated in Excel 2016 (Microsoft Office). Two time-cohorts were defined based on the Banff classification iteration used at our centre: 2015–2018 (which used Banff 2015) and 2019–2022 (which used Banff 2017). These cohorts were defined to reflect the Banff iterations that were generally used during these time periods. Additional variables were calculated as binary fields (namely C4d where C4d>0; MVI where MVI = $g + ptc \geq 2$) or as numerical fields (MVI score = $g + ptc$). The Banff '97 recommendations for adequacy(33) of ≥10 glomeruli and ≥2 arteries were not used as the number of arteries were too infrequently recorded (Supplementary Table 1). As such, the arterial requirement was dropped for adequacy and all biopsies with glomeruli were assessed.

The "calculated diagnoses" were determined using an Microsoft Excel-based ('computer-based') tool created by the authors, using conditional data retrieval and logic functions which implemented Banff 2022 criteria (link provided at end). The purpose was to assess whether the new criteria would impact rates of diagnosis (i.e. reclassify 'discordant' cases), while also acting as an audit of the laboratory's data capture practices; it was not a reflection or judgement of pathologist performance in historic reports. The agreement and discordance subsequently reported thus represent points of interrogation and assessment of the created tool.

Only the categories AMR, TCMR and suspicious (borderline) for acute TCMR (herewith "borderline rejection") were assessed. No retrospective calculations using Banff 2015 (used in 2015–2018) and Banff 2017 (used in 2019–2022) were performed. Biopsies lacking requisite Banff lesion scores were excluded from diagnostic calculations.

Data were analysed in RStudio (Version 2024.12.1+563) and IBM SPSS (version 30.0). Population descriptive statistics and temporal trends were assessed both at a biopsy and patient level using the Chi-square- and ANOVA-tests. Diagnostic agreement (reported vs. calculated diagnosis) was evaluated using Cohen's κ; cases that were reclassified by Banff 2022 (where the reported and calculated diagnoses were discordant) were tested with McNemar's test. Univariate logistic regression modelled various predictors (C4d, C4d Intensity (i.e. the Banff lesion score), MVI, and the calculated MVI score) on the dichotomous outcome of DSA (positive/negative). Model fit used Nagelkerke R^2; classification accuracy was assessed at a 0.5 probability threshold. Significance was set at $p<0.05$. Receiver operator curves (ROC) were generated to determine discriminative ability (Area under curve [AUC] with 95% CIs), with sensitivity and specificity calculated from the 2 x 2 tables. Only cases with all criteria for AMR including C4d and DSA were included in this assessment. Graphical presentation was performed using RStudio (Version 2024.12.1+563) with R (version 4.2.2, R Foundation for Statistical Computing, Vienna, Austria). Bar charts comparing prevalence rates between time periods and sex groups were created using ggplot2 (v3.4.0). Timeline analysis was conducted using lubridate and ggplot2 packages. Set intersections and overlaps were visualized using UpSetR (v1.4.0).

4 Results

4.1 Demographics and biopsy characteristics

Case selection is summarized in Figure 1**.** Initial screening identified 299 biopsies from the Department of Nephrology records. After exclusion of HIV-positive and HIV-unknown cases (n=84) and erroneously identified cases (n=18), 197 biopsies were available for retrospective review, from 135 unique patients.

There was a moderate male predominance in the cohort, with 80 males (59.3%) and 55 females (40.7%). Eighty biopsies were done in females, and 117 biopsies in males.

The median age for the cohort was 38 years (IQR: 30 – 49 years). No significant difference in overall age of sexes was found.

After evaluating the total biopsies performed annually between 2015–2022, it was observed that only 4 biopsies were documented on the TrakCare system in 2015 (vs. an average of 27.6 biopsies/year for 2016–2022). This was because recording on the TrakCare system only began in September 2015 at our hospital; access to the previous laboratory information system to account for January–August 2015 was unavailable. Following the exclusion of 2015, there was a small but significant decline in the number of biopsies performed (R^2=0.765, p=0.01, ANOVA). When stratified by period, 124 biopsies (62.9%) were done between 2015–2018, and 73 (37.1%) between 2019–2022.

The mean patient age at biopsy decreased slightly but significantly (1.58-year reduction, p=0.005, ANOVA). This trend persisted when comparing the two time-cohorts, though with marginal significance (p=0.054). The sex distribution showed no significant variation throughout the study period.

Of the 135 patients biopsied, 46 patients (34.1%) underwent multiple biopsies, with multiple biopsy procedures accounting for 108 (54.8%) of the 197 biopsies, detailed in Figure 2. Of note were two patients who each had four biopsies: patient 16 had one inadequate biopsy with subsequent repeat, and did not show any persistent/recurrence trends, over one year; patient 29 was diagnosed with TCMR, with subsequent persistent borderline rejection, all with acute tubular injury, over a period of four years.

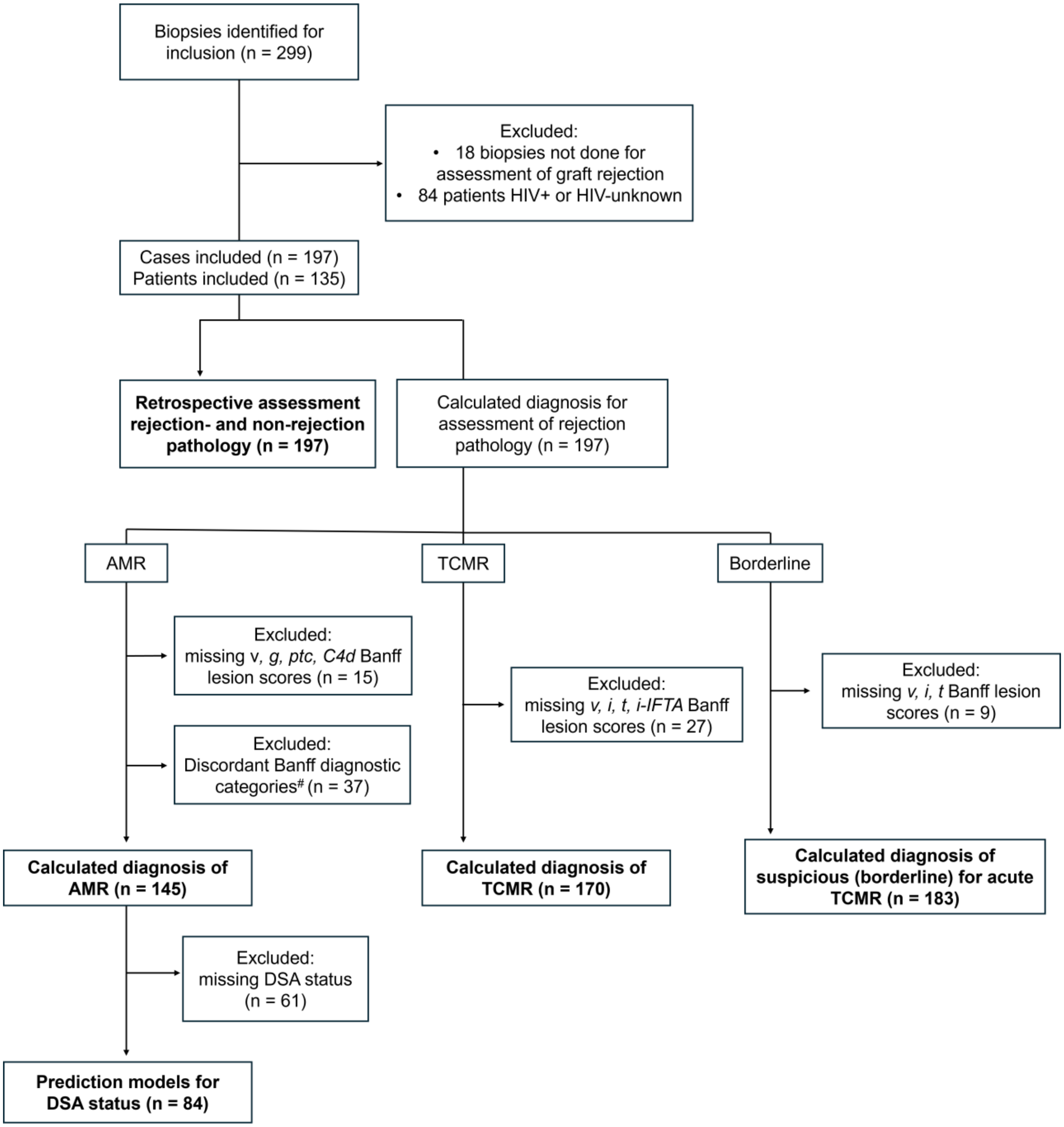

Figure 1. Flow diagram of biopsies included in this study. Emboldened blocks represent the cohorts used for investigation. The review of reported (retrospective) diagnoses included 197 biopsies/cases from 135 unique patients. For the calculated diagnoses cases were excluded based on absence of requisite Banff lesion scores: AMR utilized 145 biopsies, TCMR utilized 170, and borderline rejection utilized 183 of the original 197 biopsies. For prediction modelling, only 84 of the 197 biopsies had complete data for inclusion. Italicized letters refer to Banff lesion scores, elaborated under ‘abbreviations’.

***#** - the excluded diagnostic categories included ‘suspicious for ABMR’ discontinued after Banff 2015, and the new categories ‘probable AMR’ and ‘C4d staining with acute tubular injury’ introduced in Banff 2022.*

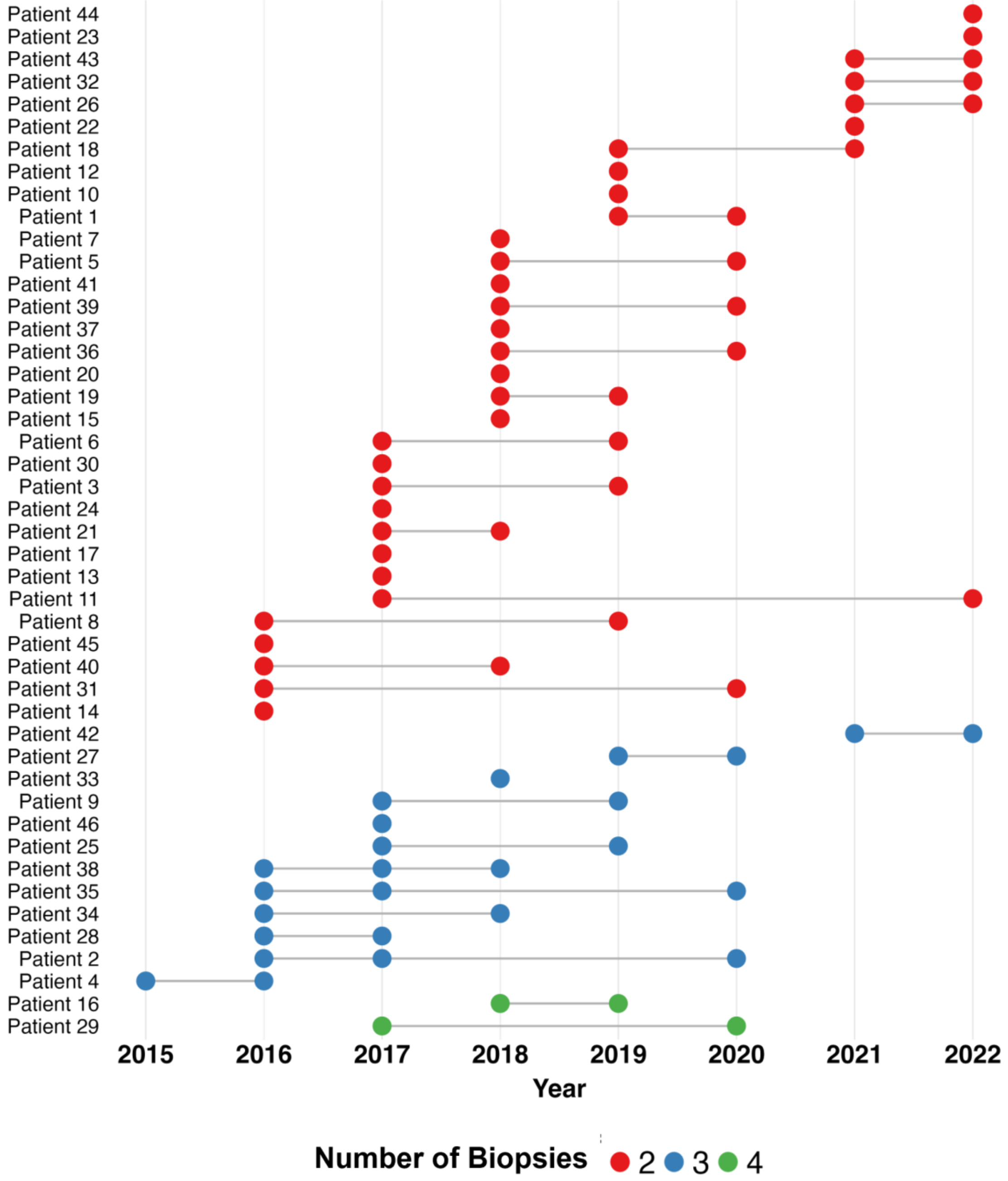

Figure 2. Biopsy timeline for patients with multiple procedures. A total of 46 patients (34.1%) had multiple for-cause biopsies, with 11 having three biopsies and 2 having four biopsies each. As our hospital does not do protocol biopsies, patients may not have many biopsies over the course of their transplant, as only symptomatic patients are targeted.

4.2 Non-rejection pathology prevalence

Non-rejection pathology is summarised in the UpSet plot in Figure 3**.** These pathologies were commonly reported within the cohort, with 124 (62.9%) of the 197 biopsies having at least one non-rejection pathology. A substantial proportion of biopsies (n=66; 33.5%) had 2 or more non-rejection pathologies, with one patient having 3-, and one patient 4- diagnoses.

Acute tubular injury was the most common non-rejection pathology reported, seen in 92 (46.2%) of biopsies, co-occurring most commonly with features of pyelonephritis, drug-induced interstitial nephritis and calcineurin inhibitor toxicity. Calcineurin inhibitor toxicity in was seen in 28 (14.2%) of cases, co-occurring with multiple other non-rejection pathologies. Pyelonephritis was recorded in 19 cases (9.6%), and similarly co-occurred with multiple other diagnoses. Features of drug-induced interstitial nephritis were seen in 14 (7.1%) of cases. Two cases (1.0%) showed BK virus infection. The other less frequent non-rejection pathologies are reported in Figure 3.

Of interest were 6 cases of ‘recurrent disease’. Three of these were in patients with systemic lupus erythematosus, 2 with mesangioproliferative glomerulonephritis (1 identified as C3 nephropathy), and 1 case of focal segmental glomerulosclerosis (of unspecified aetiology).

Notably, in this cohort, no cases of CMV nephritis, reflux nephritis or post-transplant lymphoproliferative disorder were reported.

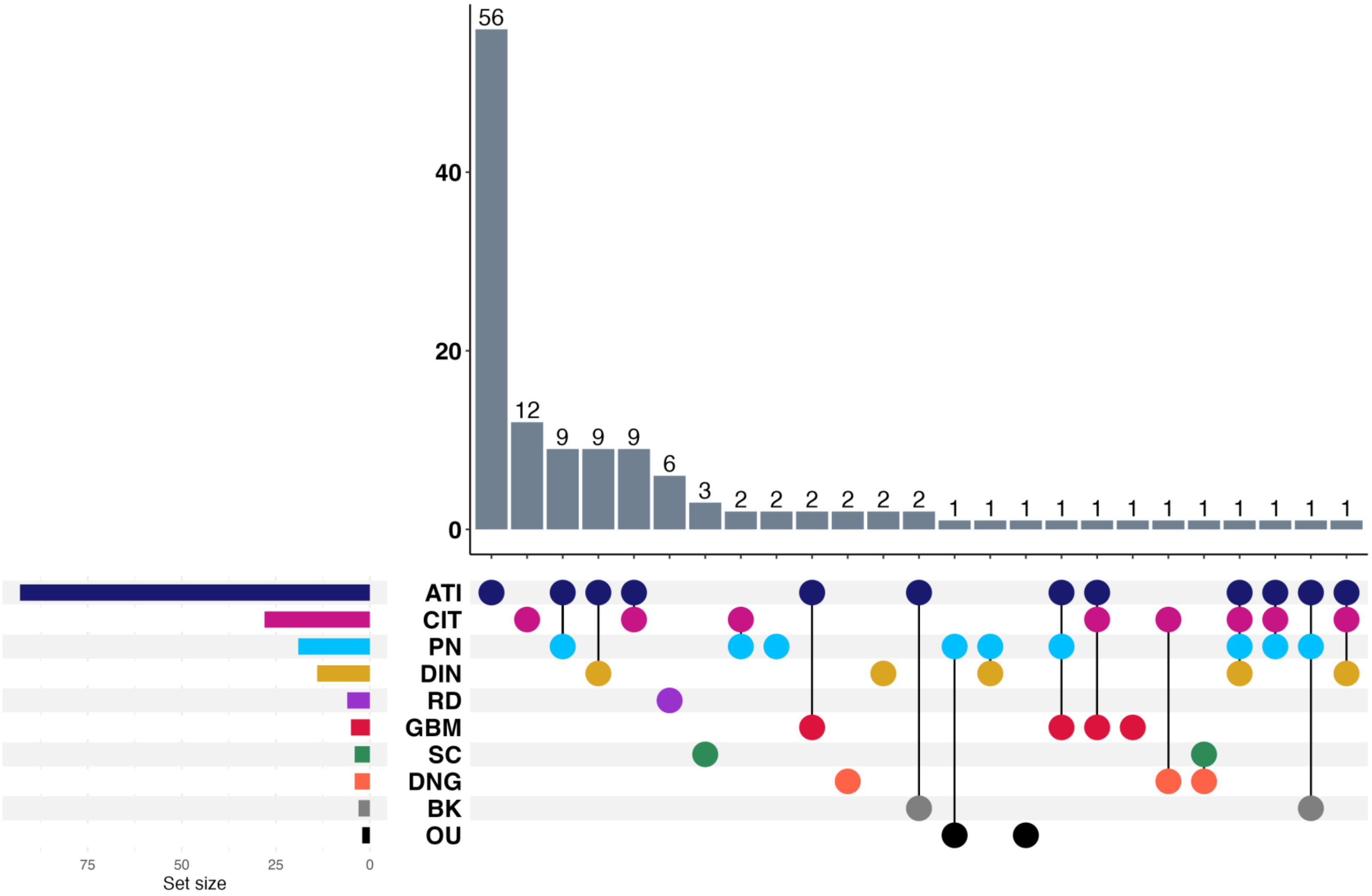

Figure 3. UpSet plot of non-rejection pathology. The set size (incidence of each of the pathologies identified) is shown on the lower left. The graph in the upper frame shows the incidence of individual- and co-occurring pathologies, with each column representing a unique pathology, or pathology conglomerate. No post-transplant lymphoproliferative disorders or CMV nephritis were identified.

***ATI:** acute tubular injury; **CIT:** calcineurin-inhibitor toxicity; **PN:** pyelonephritis; **DIN:** drug-induced nephritis; **RD:** recurrent (primary) disease;*
***GBM:** glomerular basement membrane changes; **SC:** subcapsular injury; **DNG:** de novo glomerulopathy; **BK:** BK polyomavirus infection; **OU:** obstructive uropathy*

4.3 Rejection prevalence during study period

A summary of rejection prevalence is provided in Table 1 and Figure 4. Of the 197 biopsies included, a diagnosis of AMR was reported in 15 cases (7.6%, the least frequent), with a diagnosis of ‘suspicious for AMR’ rendered in a further 39 cases (19.8%). A diagnosis of TCMR was seen in 34 cases (17.3%, the most frequent), with a diagnosis of borderline rejection seen in 28 cases (14.2%). Significant differences between time cohorts were noted for TCMR, with 28/34 cases (82.4%) occurring in 2015-2018 (p=0.01). No significant differences between time-cohorts were observed for AMR (positive or suspicious), or for borderline rejection. No proportional differences existed between sexes for any diagnoses (Table 1b).

At an individual patient level (n=135), 30 patients (22.2%) had a diagnosis of TCMR, and 24 patients (17.8%) had at least one episode of borderline rejection. Fifteen patients (11.1%) had a diagnosis of AMR, and 35 patients (25.9%) had at least one reported diagnosis of ‘suspicious for AMR’.

With regards to repeated diagnosis of rejection, 4 patients (3.0%) had a repeat diagnosis of TCMR, and 5 patients (3.7%) had at least one co-occurrence of TCMR and borderline rejection. No patients had a repeat diagnosis of AMR, however 4 patients (3.0%) had a co-occurrence of a ‘positive for AMR’ and ‘suspicious for AMR’.

Table 1: Prevalence of rejection between 2015 – 2022

a. Prevalence by time cohort[#]

Diagnosis	Time period	Positive n (%)	Negative n (%)	p (Chi-square)
AMR (Positive)	Total	15 (7.6)	182 (92.4)	
	2015-2018	12 (9.7)	112 (90.3)	p=0.155
	2019-2022	3 (4.1)	70 (95.9)	
AMR (Suspicious)	Total	39 (19.8)	158 (80.2)	
	2015-2018	24 (19.4)	100 (80.6)	p=0.839
	2019-2022	15 (20.5)	58 (79.5)	
TCMR	Total	34 (17.3)	163 (82.7)	
	2015-2018	28 (22.6)	96 (77.4)	p=0.010
	2019-2022	6 (8.2)	67 (91.8)	
Borderline	Total	28 (14.2)	169 (85.8)	
	2015-2018	19 (15.3)	105 (84.7)	p=0.561
	2019-2022	9 (12.3)	64 (87.7)	

b. Prevalence by Sex[+]

Diagnosis	Sex	Positive n (%)	Negative n (%)	p (Chi-square)
AMR (Positive)	Total	15 (7.6)	182 (92.4)	
	Females	6 (7.5)	74 (92.5)	p=0.960
	Males	9 (7.7)	108 (92.3)	
AMR (Suspicious)	Total	39 (19.8)	158 (80.2)	
	Females	18 (22.5)	62 (77.5)	p=0.431
	Males	21 (17.9)	96 (82.1)	
TCMR	Total	34 (17.3)	163 (82.7)	
	Females	12 (15.0)	68 (85.0)	p=0.488
	Males	22 (18.8)	95 (81.2)	
Borderline	Total	28 (14.2)	169 (85.8)	
	Females	12 (15.0)	68 (85.0)	p=0.794
	Males	16 (13.7)	101 (86.3)	

***AMR**: antibody-mediated rejection; **TCMR**: T cell-mediated rejection;*
***Borderline**: Suspicious (borderline) for acute TCMR*

- n (2015-2018) = 124; n (2019-2022) = 73; n (Total) = 197
+ - n (Females) = 80; n (Males) = 117; n (Total) = 197

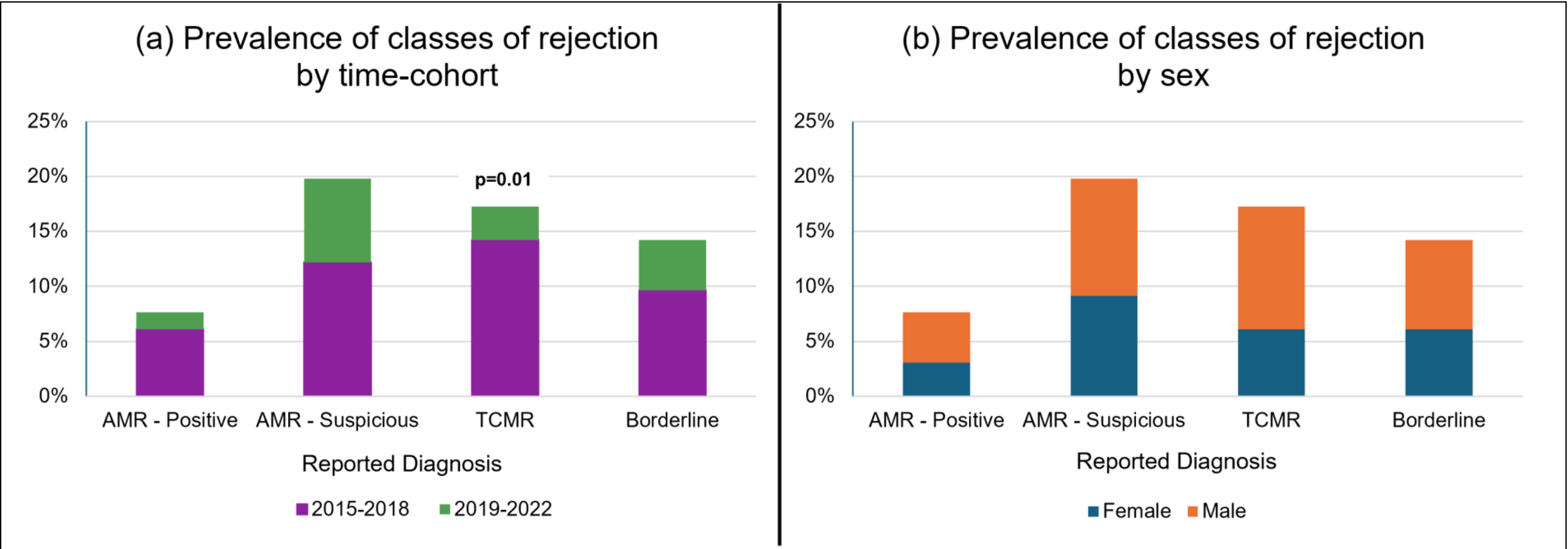

Figure 4. Prevalence of rejection over study period. (a) represents occurrence of rejection divided by time-cohort, with (b) showing division by sex. Only TCMR in (a) showed a significant difference between the two time-cohorts (p-value above graph). There were no significant differences in occurrence of rejection type by sex. 'AMR – suspicious' represents cases where insufficient diagnostic criteria for AMR were present (thus not a true diagnosis of AMR).

***AMR:** antibody-mediated rejection; **TCMR:** T-cell mediated rejection; **Borderline:** suspicious (borderline) for acute TCMR*

4.4 Agreement between reported and calculated diagnoses

The agreement between reported- and calculated diagnoses (using the Banff 2022 criteria) was evaluated for AMR, TCMR and Borderline rejection, using the Excel-based tool developed by the authors. Table 2 provides a summary of the findings. For clarity, the reader is directed to the Banff Foundation's criteria for rejection(12).

Using the Excel-based tool, AMR initially showed the lowest overall agreement of 72.9%. However, diagnostic sub-categories were not consistent over the study period and the Banff iterations used (Supplementary Table 2). Among the discordant cases, 30 cases were reported either positive or suspicious for AMR with no calculated diagnosis. Of these, the category of 'suspicious' predominated: 17 cases were diagnosed in the 2015-2018 cohort (where 'suspicious for ABMR' was an available diagnostic category by Banff 2015, but discontinued thereafter), and another 7 in the 2019-2022 cohort (where 'suspicious' was issued as a descriptive term/precautionary diagnosis and not an assertion of AMR, in the absence of all required features). Thus, only 6 cases diagnosed 'positive' were discordant, 5 of which were diagnosed in 2015-2018. Four were likely explained by insufficient *C4d* lesion scores, and two had co-existing acute TCMR, the higher thresholds for *g* and *ptc* lesion scores were not met.

Of the initial 17 cases of AMR that were calculated as positive but had no reported histologic diagnosis of AMR, 12 were accounted for by the new diagnostic categories 'probable AMR' and 'C4d staining with acute tubular injury', thus leaving only 5 cases (3 in 2015-2018, and 2 in 2019-2022) that were additionally identified through the calculated diagnosis (Supplementary Table 2). Review of these showed that of the 2 cases the tool labelled 'Active AMR', 1 had concurrent non-rejection inflammatory pathology, and 1 case (0.7%) may have been additionally detected by the tool. Of the 3 cases labelled 'chronic (inactive) AMR' by the tool, 1 showed non-rejection inflammatory pathology, 1 showed recurrent disease, and 1 case (0.70%) may have been additionally detected by the tool.

Following the exclusion of these cases, 145 cases were included for agreement assessment of AMR. AMR had an overall agreement of 92.41%, with good agreement between reported and calculated diagnoses ($\kappa=0.730$, $p<0.001$), which was slightly higher for the 2019-2022 group ($\kappa=0.809$, $p<0.001$) than the 2015-2018 group ($\kappa=0.681$, $p<0.001$). No significant differences were identified in distribution of the 11 discordant/reclassified cases overall, nor when stratified by time cohort.

Table 2: Agreement and discordance between reported and calculated diagnoses

a: Overall assessment of concordance

Diagnosis	Time period	Total Cases	Reported Positive	Calculated Positive	Concordance n (%)	Cohen's κ (p)
AMR	Total	145	25	24	134 (92.41)	0.730 (p<0.001)
	2015 - 2018	91	16	14	83 (91.21)	0.681 (p<0.001)
	2019 - 2022	54	9	10	51 (94.44)	0.809 (p<0.001)
TCMR	Total	170	29	36	157 (92.35)	0.753 (p<0.001)
	2015 - 2018	104	23	26	100 (96.15)	0.892 (p<0.001)
	2019 - 2022	66	6	11	57 (86.36)	0.400 (p>0.001)
Borderline	Total	183	27	46	151 (82.51)	0.455(p<0.001)
	2015 - 2018	117	18	29	99 (84.62)	0.519 (p<0.001)
	2019 - 2022	66	9	17	52 (78.79)	0.345 (p=0.003)

b: Breakdown of discordant (reclassified) results

Diagnosis	Time period	Total Cases	Reported+/ Calculated-	Reported-/ Calculated+	Discordance n (%)	p (McNemar's)
AMR	Total	145	6	5	11 (7.59)	p=1.000
	2015 - 2018	91	5	3	8 (8.79)	p=0.727
	2019 - 2022	54	1	2	3 (5.56)	p=1.000
TCMR	Total	170	3	10	13 (7.64)	p=0.092
	2015 - 2018	104	1	3	4 (3.85)	p=0.625
	2019 - 2022	66	2	7	9 (13.64)	p=0.180
Borderline	Total	183	7	25	32 (17.49)	p=0.002
	2015 - 2018	117	4	14	18 (15.38)	p<0.001
	2019 - 2022	66	3	11	14 (21.21)	p=0.057

Diagnostic sub-categories for of TCMR and borderline rejection were consistent over Banff iterations used during the study period.

TCMR showed fair to good agreement between reported and calculated diagnoses ($\kappa=0.753$, $p<0.001$), with an overall agreement of 92.35%. Concordance was significantly poorer for TCMR in 2019-2022 ($\kappa=0.400$, $p<0.001$). However, overall discordance was not significant between time-cohorts ($p=0.092$). Three cases were reported as TCMR but were not positive by calculation: 2 of these did however include descriptive terms suggesting TCMR, but were not outright in their diagnostic line; in the other case a transcription error was identified in the report. All 10 cases reported as negative for TCMR but were positive with the calculator could be explained: 5 cases showed features of AMR as the primary pathology, 2 showed disease recurrence, 2 showed evidence of a drug-induced nephritis, and 1 developed a *de novo* glomerulopathy.

Of interest was the large significant discordance in cases of borderline rejection. The overall agreement was 82.51% ($\kappa= 0.455$, $p < 0.001$). Discordance was 17.49%, with significant differences in discordant pairs ($p=0.002$), driven by 2015-2018 ($p<0.001$).

Seven cases were reported positive for borderline rejection but were not positive by calculation. Of these, 3 in 2015-2018 were indeed correct by Banff 2015 criteria (which changed in 2019), 2 used incorrect terminology ('borderline rejection' instead of 'suspicious for ABMR', a term available in Banff 2015 but incorrectly captured), 1 had a '*v*' score that was in a comment explained to be due to recurrent primary disease (and so effectively regarded as '*v0*', compatible with borderline rejection). One case (0.55%) did not meet Banff criteria for the period (Banff 2017) nor Banff 2022 and likely represents a false positive diagnosis.

Of the 25 cases reported negative but calculated positive, 17 could be explained by non-rejection pathology (5 showed acute tubular injury; 6 showed pyelonephritis; 3 showed calcineurin inhibitor toxicity; 1 showed drug induced nephritis; 1 showed disease recurrence; 1 BK virus infection was reported). There were 2 transcription errors, 1 biopsy scored in non-diagnostic scarred areas (but given Banff lesion scores), and 1 biopsy that was reported as insufficient, but scores reported. There were thus 4 cases, 1 reported 'unclear if rejection or secondary cause', and 3 (1.64% of the 183 cases of borderline rejection) that appear to be additionally detected.

4.5 C4d as a predictor of DSA status

The outcomes of the four binary logistic regression models are summarized in Supplementary Table 3. These analyses revealed C4d was the most robust predictor of DSA status.

In these univariate analyses, binary C4d (positive/negative) showed an odds ratio (OR) of 7.97 (Confidence interval (CI): 1.67-38.01, p=0.009) with weak to moderate discrimination (area under curve (AUC)=0.623). This model showed high specificity (95.0%) but poor sensitivity (29.5%). C4d intensity showed similar results, with an OR of 4.99 (CI: 1.26-19.77, p=0.022), weak to moderate discrimination (AUC=0.627), with the same sensitivity, specificity, and overall accuracy (given calculation from the same 2 x 2 table values). The Nagelkerke R^2 showed that both C4d and C4d intensity had a very modest (values >0.1) but significant influence on DSA status.

Additionally investigated as a possible predictor of DSA status was microvascular inflammation. Neither the binary MVI nor the MVI Score models performed better than chance (AUC = 0.539 & 0.536), with non-significant p-values.

5 Discussion

This retrospective cohort study evaluated the prevalence of renal allograft rejection in HIV-negative patients at Groote Schuur Hospital and investigated diagnostic tools to aid in accurate and streamlined diagnoses.

5.1 Demographics and biopsy characteristics

In keeping with other South African cohorts from Cape Town(4, 31) and Johannesburg(34), our patients showed a male predominance, and was a younger population than international studies. This persistent sex disparity may reflect socioeconomic access inequalities, requiring further qualitative research.

The small but significant decrease in the number of biopsies is difficult to interpret in this study, given that our study lacks the HIV-positive population biopsied between 2015 and 2022, a group that comprised ~10% of the originally identified population (28/281 biopsies done for renal rejection) and may thus likely influence trends. However other contributing factors may include changes in the geographic area serviced by our hospital (with diversion of work from the Southern Cape to other centres between 2017–2021), as well as the COVID-19 pandemic where less biopsies were performed.

5.2 Comparison of rejection and non-rejection pathology with international cohorts

The overall prevalence of AMR, TCMR and borderline rejection in our study were consistent with international cohorts(7, 16, 19). As in other studies, TCMR was the most prevalent form of rejection noted in our HIV-negative cohort.

A comparison of our findings to two South African cohorts is shown in table 3. Despite relatively small sample sizes, all studies involved patients managed by the Department of Nephrology at Groote Schuur Hospital, minimizing confounders such as variations in treatment protocols and resource allocation.

Wearne *et al(31)* focused only HIV-positive patients – a group excluded from our analysis – but which remains comparable given the shared setting. When considering individual biopsies as cases, our study showed an increased prevalence of AMR, TCMR and borderline rejection, likely because their protocol biopsies (none of which were reported to show rejection) likely diluted their reported prevalences. Since our cohort were not subjected to protocol biopsies, this dilution was absent. This confounder was helped when cases were defined by individual patients, where their HIV-positive cohort showed increases in AMR and borderline rejection compared to our study, a finding likely keeping with the known higher rates in HIV-positive cohorts(35). The shorter follow-up (≤52 weeks) may explain the lack of similar trend in TCMR, and is an important factor that may under-estimate rejection in their study.

Table 3: Comparison of the present study to two other cohorts from our hospital

Study (ref.)	Biopsy level				Patient level			
	n	AMR n (%)	TCMR n (%)	Borderline n (%)	n	AMR n (%)	TCMR n (%)	Borderline n (%)
Current study	197	15 (8)	34 (17)	28 (14)	135	15 (11)	30 (22)	24 (18)
Davidson *et al* (4)#	-	-	-	-	198	* (11–36)	* (18–37)	* (34–48)
Wearne *et al* (31)	179	9 (5)	12 (7)	25 (14)	50	9 (18)	9 (18)	16 (32)

***AMR**: antibody-mediated rejection; **TCMR**: T cell-mediated rejection; **Borderline**: Suspicious (borderline) for acute TCMR*

- *Davidson* et al *did not report overall prevalences, but rather prevalences at 1 month, 1 month – 1 year, and 1 year – 5 years. Reported are the minimum and maximum ranges for these time frames. Number of cases was not reported.*

Although Davidson *et al(4)* did not explicitly report overall prevalences for their study, our findings align with the lower end of their reported rejection ranges, except for our findings for borderline rejection, which was significantly lower. Their study used Banff 2007 criteria, which possesses similar diagnostic criteria for AMR and TCMR to the Banff iterations used in our study cohort, but at least for the 2019-2022 time-cohort, criteria for borderline rejection were different. This, and the different time periods assessed, may suggest that differences in prevalence reflect improvements in patient care at our hospital. Future studies should incorporate clinical and histologic data to identify specific factors leading to this decline.

In terms of non-rejection related pathology, our cohort have a comparable overall prevalence compared to Kenyan(36) and Indian(37) cohorts. Of interest is our high rate of acute tubular injury, which is ~2–3x higher than the referenced cohorts. Although the nature of the biopsy in these groups is not stated (protocol vs. for-cause), this increased prevalence may speak to our hospitals for-cause biopsy strategy, where clinically ill patients are biopsied, with otherwise stable patients not biopsied; protocol biopsies in well patients with no co-existing pathology may act to dilute these rates. Calcineurin inhibitor toxicity in our cohort was similar to the Indian(37) and other western cohorts(38). Of interest was the absence of post-transplant lymphoproliferative disorder, and CMV nephritis in our cohort: our relatively small sample size likely explains their absence, given the rarity of these two entities. However, it is a good reminder for our practice to be aware of these entities, so as to not miss the rare presentation.

5.3 Agreement of the Banff 2022 criteria and utility of a computerized diagnostic tool

Comparing our cohorts reported histologic diagnoses to the diagnoses calculated by our simple Excel-based calculator provided a valuable retrospective audit, Banff iteration comparison, and a promising prospective role in our laboratory.

The intentions behind an automated tool were to assess, firstly, if there were any significant changes to diagnoses when the Banff 2022 criteria were applied, and if so, what these changes were. Secondly, the tool was intended to check whether pathologists correctly translate parameters to arrive at the correct final diagnosis. The tool was created acknowledging the difficulties in use of the Banff framework, and not as a retrospective judgement.

In the case of AMR, the tool helped identify an important consideration when diagnosing AMR: the presence of TCMR upgrades the required Banff lesion scores to diagnose concurrent AMR. In 2 cases, this was not apparent from the report, and the tool helped identify both the reporting error and the likely correct diagnosis. It further acted to remind of the critical role of C4d staining in the diagnosis of AMR; apart from 'chronic AMR' and the new categories 'Microvascular inflammation/injury (MVI), DSA-negative and C4d-negative' and 'Probable AMR', AMR (moreover clinically actionable AMR) requires C4d staining to be present. In cases without C4d staining, a diagnosis of AMR must be carefully re-assessed, highlighted by the 4 possible false-positives identified in our cohort.

On review of the discrepancies in TCMR in reported vs. calculated cases, an important base truth was demonstrated: Banff lesion scores alone do not indicate rejection, and the clinical history, histology and scoring must be assessed as a whole. In the 10 discordant cases that were reported negative but calculated positive, it was noted that the tool was not discriminating cases with features of AMR or recurrent primary pathology (the latter as this was not often recorded in the diagnostic line). This is an important limitation identified, which may be addressed in future iterations of this tool.

Suspicious (borderline) for acute TCMR showed the lowest concordance. Although a small percentage were indeed due to Banff criteria changes (3 cases, 1.64%), 1 false positive was identified. An additional 25 cases (13.7%) were identified by the tool. From the available reported histologic data, it was clear that all but 3 cases (1.64%) had other co-existing non-rejection inflammatory processes which better explained the histopathology/Banff lesion scores, again underlying the importance of contextualizing the Banff lesion scores. This is an important limitation in our tool, and one with important clinical impact, as false positive results may lead to inappropriate treatment. Although the 2020 KDIGO guidelines recommend treating suspicious/borderline acute rejection (grade 1 evidence), the overall quality of the evidence is graded as "very low quality of evidence". In a resource-limited setting such as ours, it is sometime difficult to justify treatment where "the estimate of effect is uncertain, and often will be far from the truth"(13, 14). This further underscores the guiding, and not diagnostic, role that our tool plays.

The review also acted as an internal quality control, identifying 3 transcription errors and 2 cases where incorrect terminology was applied. In both cases, an incorrect diagnosis for the patient may result, underscoring the importance of case review. It is possible that these could have been avoided with the use of our diagnostic tool, which is designed to give the updated terminology based on the Banff 2022 criteria, and acting as a final guide for diagnostic reporting.

Lastly, the tool demonstrated and reminded how complex and convoluted applying the Banff criteria can be. A category may have many inter-connected requirements and exceptions for each category, and occasionally categories are inter-dependent (active AMR is a good example of this(22)). Making a diagnosis is not only a challenge histologically, but even with accurately assessed histologic Banff lesion scores, compiling them into a diagnosis can be a confusing process. The tool, although not a true diagnostic calculator, aims to function as a prompt for the general pathologist to avoid misinterpretation or missed diagnostic considerations.

A beta model using a graphics user interface asking for additional confounders is in development at the time of submission and will hopefully further contribute to diagnostic guidance.

5.4 Predictors of DSA positivity in our setting

C4d has been emphasized by the Banff 2022 framework as a vital component for the diagnosis of AMR. The recent update highlights that when C4d is positive, even where MVI thresholds are not met, AMR can be diagnosed (reference (22); see reproduction in Supplementary Figure 1). Given the role of C4d as a surrogate marker for DSA and considering the difficulties in assessing DSA in our setting, evaluating the performance of C4d at our centre was critically needed. This was additionally important to aid clinical colleagues in understanding the critical role of this marker and decrease sole reliance on DSA, given that other robust diagnostic molecular markers are still under investigation.

The univariate modelling of four predictive factors demonstrated that binary C4d status and numeric C4d intensity, although modest in power, were in line with the known role of C4d as a surrogate for DSA status(28, 29, 39). Microvascular inflammation showed no predictive power in our cohort.

The high specificity of C4d immunohistochemistry in detecting DSA negative cases at our hospital may prove a useful cost-saving tool. Given the difficulties with DSA, one of which is the limited volume that can be processed, using C4d to guide decision making decreases the pressure placed on the DSA service. A small proportion (5%) of cases may show false C4d positivity (for example, in ABO-incompatible transplants(28)). However given that the managing nephrologists know the transplant type, and given their clinical suspicion and the other histologic features, these additional considerations would, in this 5%, inform the decision to test DSA; C4d status and intensity are thus a suggestive tool, not a rule in/rule out beacon.

Of some concern is the poor sensitivity of our C4d immunohistochemistry at our hospital, which would miss ~70% of true DSA-positive cases. This may be affected by many factors in the chain of biopsy handling, such as variation in tissue fixation times, issues in tissue processing, as well as the immunohistochemical protocol used by our laboratory. These assumptions are supported by the significant (>0.1) but low Nagelkerke R^2, emphasizing that much of the variance seen is influenced by factors not identified by our study, which require further investigation.

One contributing factor may be the known poorer sensitivity and specificity of C4d immunohistochemistry (used at our hospital), versus C4d immunofluorescence(28, 30, 39). Immunofluorescence has also been shown to have a stronger agreement between pathologists(39), and is a more robust means of C4d assessment. It does, however, have technical requirements (additional tissue examined at the time of biopsy by dissecting microscope, specialized storage media, additional staining techniques, etc). It is worthy of future research to investigate this modality at our hospital, as a more accurate C4d identification may aid to decreased long-term costs and faster patient intervention, with hopefully decreased graft failure and better patient outcomes.

5.5 Limitations

One of the primary limitations in this study was the lack of sufficient clinical information. Although age and sex are obtainable from the laboratory information system, more detailed clinical histories are often not available on request forms. Furthermore, the aetiology of the antecedent ESKD was largely unknown, which may have import for long term graft survival, given varying risks of recurrent disease. Knowledge of this and factors such as ischaemic time may help to understand, for example, the high rate of ATI seen in this study.

In addition, our study did not know the type of donor/allograft (e.g. cadaveric donor, living relative donor, donation after brainstem death, etc) and ABO compatibility status. Previous studies have shown that these have an effect on long term graft survival in our setting(4), and replicating this with a later cohort may give valuable reflective clinical insights for the clinical Nephrology team. Furthermore, ABO-incompatible grafts may show C4d staining representing graft accommodation(28), which in the absence of this knowledge, could have been misinterpreted by our compuer-based as rejection.

Our computer-based tool had limitations: it was not built to account for the non-specificity of some Banff lesion scores, which can occur due to non-rejection pathology, and require the pathologist to contextualize the findings. Thus, future version will need more user input to try and account for this.

Lastly, our cohort is relatively small, with an even smaller portion available for predictive modelling. Future research focusing on larger cohorts, and perhaps collaboration with other local and sub-Saharan centres, would give more robust statistics for C4d as a predictor of DSA status, an invaluable tool in a resource limited setting.

6 Conclusion

This study provides insights into the diagnostic landscape of renal allograft rejection in a South African cohort. The study further demonstrates the utility of the Banff 2022 criteria, and the role of a computer-based diagnostic tool in a resource-limited setting. The prevalence of various types of rejection appears stable when compared to previous cohorts in our setting. While our rates of rejection- and non-rejection pathology appear high, these are likely inflated due to the for-cause biopsy practice (and not protocol biopsy) at our hospital.

Our analysis demonstrates that while the updated Banff framework offers some improvement in diagnostic standardization. Although not acting as a pure diagnostic tool, the tool could act as an important adjunct for guidance of the general pathologist, and ultimately helped identify possible additional cases, a minority representing potential new diagnoses. This underscores that such tools serve best as adjuncts to, rather than replacements for, expert pathological assessment.

C4d immunohistochemistry emerged as a valuable, albeit imperfect, surrogate for DSA status, with high specificity but limited sensitivity. This underscores its potential to guide early therapeutic decisions when DSA results are pending, though its clinical application must account for false negatives. Future studies should explore whether transitioning to C4d immunofluorescence could enhance detection rates, given its superior performance in other populations.

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8 Article acknowledgements

Contributions

Jarryd Lunn:	Conceptualization, data collection and curation, methodology, data analysis, project administration, writing of final submission
Nadia M. Ikumi:	Data analysis and data representation, editing, supervision
Dharshnee R. Chetty:	Project administration, editing, supervision
Joanne Lu:	Data tool design, data collection and curation
Brendon Price:	Conceptualization, project administration, editing, supervision

Conflict of interest

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Funding

No funding was obtained for this project

Data availability statement

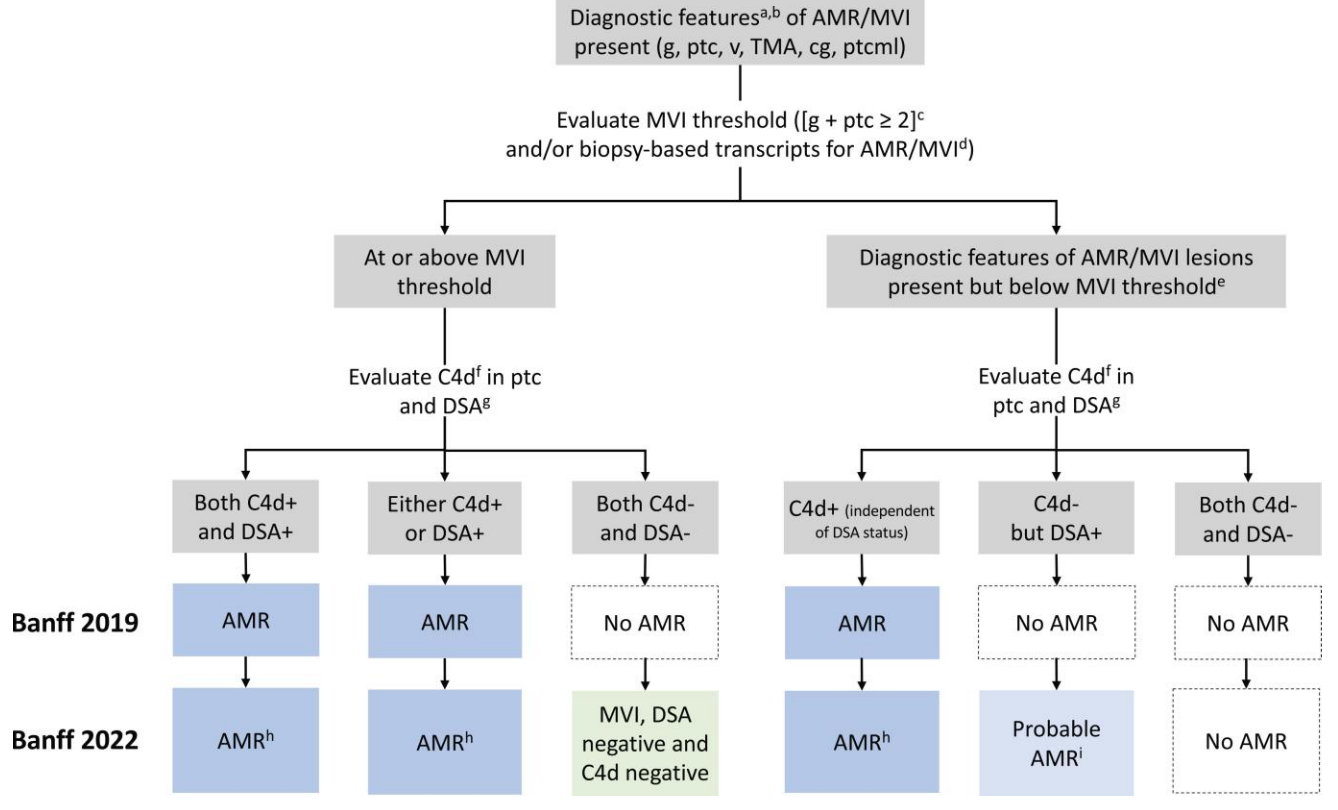
Raw data were generated at Groote Schuur Hospital. Derived data supporting the findings of this study are available from the corresponding author, [BP], on reasonable request.
Data capture tool available at link: Data capture instrument

9 Appendices and supplementary data

9.1 Supplementary figures

Supplementary Figure 1: Flowchart of the Banff 2022 Classification for Category 2: Antibody-mediated rejection and microvascular inflammation/injury (AMR/MVI)

directly reproduced from (ref 22)



a. Other lesions can be observed in AMR and strengthen the diagnosis but are not diagnostic by themselves: arterial intimal fibrosis (cv) of new onset, excluding other causes; leukocytes within the sclerotic intima favour chronic AMR if there is no prior history of TCMR; acute tubular injury, in the absence of any other apparent cause.
b. Definitions of “diagnostic features of AMR/MVI”: g>0 in the absence of glomerulonephritis; ptc > 0 in the absence of acute TCMR or borderline (suspicious) for acute TCMR; v>0; acute thrombotic microangiopathy (TMA) in the absence of any other cause; cg>0 by LM, or EM if available, if no evidence of chronic TMA and if absence of recurrent or de novo glomerulonephritis; ptcml = seven or more layers in 1 cortical peritubular capillary and 5 or more layers in 2 additional capillaries, avoiding portions cut tangentially by EM, if available.
c. [g + ptc ≥ 2] in the absence of recurrent or de novo glomerulonephritis. If borderline (suspicious) for or acute TCMR, or infection are present, [g + ptc ≥ 2] is not sufficient and Banff lesion score g≥1 is required.
d. Biopsy-based transcript diagnostics for AMR/MVI above a defined threshold, if thoroughly validated for use a substitute for AMR/MVI and available.
e. In cases of MVI below threshold, biopsy-based transcript diagnostics can be applied, if thoroughly validated for use as a substitute for AMR/MVI and available.
f. C4d deposition should be evaluated in peritubular capillaries and vasa recta (C4d positive = C4d2 or C4d3 by IF on frozen sections, C4d >0 by IHC on paraffin sections).
g. If thorough testing for DSA (anti-HLA or other specificity) has not yet been performed, this should be done, following the STAR guidelines. Detection of non-HLA antibodies (including ABO antibodies in ABO-incompatible transplantation) can be used as serologic Banff criterion for diagnosis of AMR, if the testing protocols are sufficiently standardized and clinically validated for the appropriate clinical context. At present, no non-HLA antibodies (apart from ABO antibodies) have been validated sufficiently for inclusion into the routine clinical classification of kidney transplant biopsies.
h. Upon diagnosis of AMR, further differentiation of disease stage is as follows. Active AMR: presence of only active features (including C4d positivity) (cg=0; ptcml=0); Chronic active AMR: presence of both active (including C4d positivity) and chronic (cg>0 and/or severe ptcml) features.
i Cases with “Probable AMR” and histological chronic lesions (cg or ptcml) can be labelled as “chronic AMR”. For these cases, prior documented diagnosis of active or chronic active AMR, or documented prior evidence of DSA, also count as DSA positivity.

9.2 Supplementary tables

Supplementary Table 1: Assessment of cohort using Banff ’97 criteria (ref 33)

Total biopsies	Glomeruli ≥10	Arteries with elastic lamina (AEL) ≥ 2	Glomeruli ≥10 AND AEL ≥ 2	Glomeruli ≥10 AND AEL ≥2 AND dx AMR
n = 197	n = 138	n = 47	n = 36	n = 6

***AEL**: arteries with elastic lamina; **AMR**: antibody-mediated rejection*

Supplementary Table 2:
Breakdown of discordant categories between Banff iterations

Reported+/Calculated- (n=30)		Reported-/Calculated+ (n=17)	
2015 - 2018	22	2015 - 2018	8
Reported "Positive"	5	Active AMR	1
Reported "Suspicious"	17	Chronic (inactive) AMR	2
		Probable AMR	2
		C4d staining with acute tubular injury (ATI)	3
2019 - 2022	8	2019 - 2022	9
Reported "Positive"	1	Active AMR	1
Reported "Suspicious"	7	Chronic (inactive) AMR	1
		Probable AMR	3
		C4d staining with acute tubular injury (ATI)	4

***AMR**: antibody-mediated rejection; **ATI:** acute tubular injury*

Supplementary Table 3: Binary logistic regression models for prediction of DSA status

Model	Predictor	OR (95% CI)	p-value	Nagelkerke R^2	AUC (95% CI)	Sensitivity	Specificity	Accuracy
1	C4d	7.97 (1.67-38.01)	0.009	0.143	0.623 (0.50-0.74)	29.5%	95.0%	60.7%
2	MVI	0.70 (0.28-1.76)	0.445	0.009	0.539 (0.41-0.66)	72.7%	35.0%	54.8%
3	C4d Intensity	4.99 (1.26-19.77)	0.022	0.175	0.627 (0.51-0.75)	29.5%	95.0%	60.7%
4	MVI Score	0.89 (0.66-1.21)	0.469	0.008	0.536 (0.41-0.66)	72.7%	35.0%	54.8%

CI: *confidence interval;* ***AUC****: area under curve*

9.3 MMed research course attendance confirmation

UCT CLINICAL RESEARCH CENTRE
every step of the study

Ms Annemie Stewart
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24 Jan 2024

To Whom it May Concern

Statement confirming MMeds Research Methods

This letter confirms that **Dr Jarryd Lunn attended** the **Clinical Research Centre's MMeds Research Methods Course on the 23rd Sep and 1st Oct 2021**

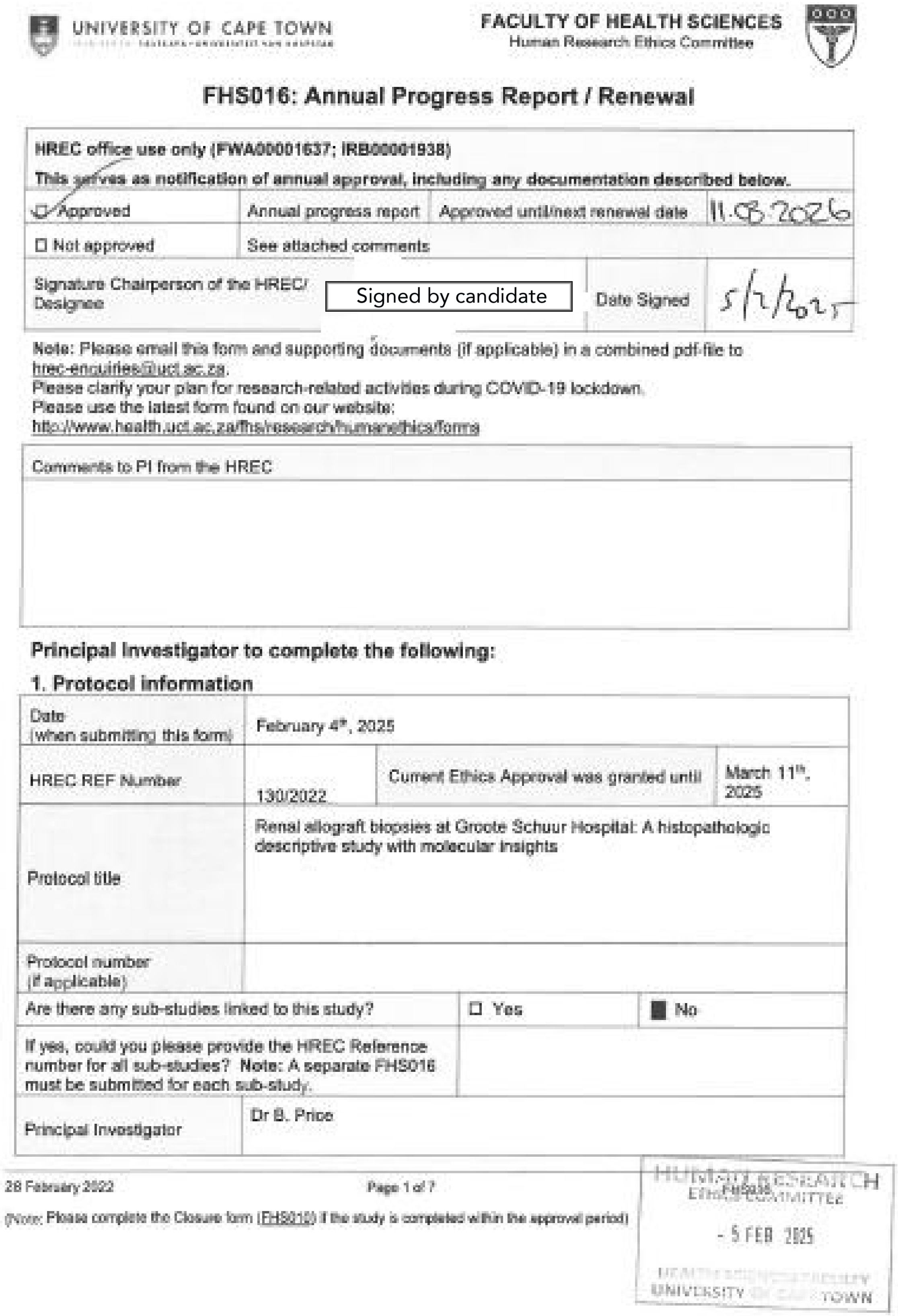
If you have any questions or concerns, please do not hesitate to contact me on **021 406 6498** or **072 408 0459**.

Best wishes

Signed by candidate

Annemie Stewart
Operations Manager
Clinical Research Centre (CRC)
UCT Faculty of Health Sciences

9.4 Ethics approval letter



UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938) **This serves as notification of annual approval, including any documentation described below.**			
☑ Approved	Annual progress report	Approved until/next renewal date	11.03.2026
☐ Not approved	See attached comments		
Signature Chairperson of the HREC/ Designee	Signed by candidate	Date Signed	5/2/2025

Note: Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za.
Please clarify your plan for research-related activities during COVID-19 lockdown.
Please use the latest form found on our website:
http://www.health.uct.ac.za/fhs/research/humanethics/forms

Comments to PI from the HREC

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	February 4th, 2025		
HREC REF Number	130/2022	Current Ethics Approval was granted until	March 11th, 2025
Protocol title	Renal allograft biopsies at Groote Schuur Hospital: A histopathologic descriptive study with molecular insights		
Protocol number (if applicable)			
Are there any sub-studies linked to this study?	☐ Yes	■ No	
If yes, could you please provide the HREC Reference number for all sub-studies? Note: A separate FHS016 must be submitted for each sub-study.			
Principal Investigator	Dr B. Price		

28 February 2022

Page 1 of 7

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

HUMAN RESEARCH
ETHICS COMMITTEE
- 5 FEB 2025
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

9.5 List of variables and data capture Instrument

The list of variables included: age/date of birth; sex; HIV status; biopsy date; biopsy adequacy features; all Banff lesion scores; all non-rejection pathologies; DSA status.

Given the relative complexity of code of the data capture tool, the file containing the instrument and complete list of variables is available at the following link: Data capture instrument

9.6 Instructions for authors (Comprehensive)

Text directly copied from: https://jcmsa.org.za/index.php/jcmsa/pages/view/submission-guidelines#part_2

Style and format

File format
Manuscript files can be in the following formats: DOC, DOCX, or RTF. Microsoft Word documents should not be locked or protected.
LaTeX documents (.tex) should be converted into Microsoft Word (.doc) before submission online.
Rich Text Format (RTF): Users of other word processing packages should save or convert their files to RTF before uploading. Many free tools are available that will make this process easier.

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Manuscripts should adhere to the author guidelines of the journal. There are restrictions on word count, number of figures, or amount of supporting information.

Font
Use a standard font size and any standard font family.

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Do not use the font named ‘Symbol’. To add symbols to the manuscript, use the Insert → Symbol function in your word processor or paste in the appropriate Unicode character. Refer to our AOSIS house style guide on mathematical and Unicode font guidelines.

Headings
Ensure that formatting for headings is consistent in the manuscript. Limit manuscript sections and sub-sections to four heading levels. To avoid confusion during the review and production process, ensure that the different heading levels used in your work are visually distinct from one another. The simplest way to achieve this is to use different font sizes and/or a combination of bold/italics for different heading levels.

Keywords
Identify eight keywords that represent the content of your manuscript and are specific to your field or sub-field. Test your keywords: when you enter your keywords into the various journal and academic databases like Google Scholar, do the results include papers similar to your topic? If not, revise the terms until they do.

Layout and spacing
Manuscript text should have a 1.5 line spacing.

Page and line numbers
Include page numbers and line numbers in the manuscript file. Use continuous line numbers (do not restart the numbering on each page).

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Footnotes are not ideal. If your manuscript contains footnotes, move the information into the main text or the reference list, depending on the content.

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Manuscripts must be written in British English, according to the Oxford English Dictionary (avoid Americanisms [e.g. use ‘s’ and not ‘z’ spellings], and set your version of Microsoft Word default language to UK English). Refer to the AOSIS house style guide for more information.

Abbreviations

Define abbreviations upon first appearance in the text. Do not use non-standard abbreviations unless they appear at least three times in the text. Keep abbreviations to a minimum.

Illustrations

Illustrations fall into two categories:
Figures: Photographs, drawings, diagrams, graphs, flowcharts, maps, etc.
Tables and/or Boxes: Text and/or numbers arranged in orderly columns and rows.
Every time a Figure, Table and/or Box is presented in your manuscript, it should be referred to three times:

In a legend, which includes a number, a title, and its source. The legend is placed below a Figure and above a Table and/or Box. The source section should consist of the in-text citation, creator or owner and its year of creation, and any other attribution required as stipulated by the permission received (person and place) to reproduce.

In the body of your written manuscript. You should include an in-text citation and a sentence or two about the image explaining what it illustrates and why it is there.
As a reference entry within your reference list.

AOSIS house style

The manuscript must adhere to the AOSIS house style guide.

References

Referencing style guide

The manuscript must adhere to the Vancouver referencing style.

9.7 Turnitin report

Follows:-

FACULTY OF HEALTH SCIENCES (FHS)

Plagiarism Declaration and Supervisor Authentication

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I, the undersigned, declare that:

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Signature: ___Signed by candidate___

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by Jarryd Lunn

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Submission ID: 2646750282
File name: LNNAJR001_MMed_FINAL.docx (1.61M)
Word count: 6415
Character count: 36968

Abstract

Background: Chronic kidney disease affects millions in South Africa, with transplantation offering the best outcomes for end-stage disease. Immune-mediated rejection remains a critical challenge, diagnosed through the Banff classification, incorporating histopathology and biomarkers (C4d/DSA). In resource-limited settings, DSA testing is difficult, necessitating reliable alternatives.

Aim: This study evaluated: (1) rejection patterns at our hospital, (2) the impact of the Banff 2022 criteria with a computer-assisted tool, and (3) C4d's utility as a predictor of DSA status.

Methods: We analysed 197 for-cause biopsies from 135 HIV-negative transplant recipients (2015-2022). Historic reports and rejection- and non-rejection pathologies, Banff lesion scores and HIV/DSA status were recorded. A computerized algorithm automated Banff 2022 classification, with results compared to historic diagnoses.

Results: The cohort showed male predominance (*%) with median age 39 years. Non-rejection pathology was common (* showing at least one diagnosis). The computer tool demonstrated agreement of 92.4% for AMR/TCMR and 84.6% of borderline TCMR, but was confounded by non-rejection pathology. C4d predicted DSA positivity with 95% specificity but only 29.5% sensitivity (Nagelkerke R^2 0.14-0.18), indicating moderate utility in DSA absence.

Conclusion: The Banff 2022 criteria were additive in rejection diagnosis, with a computerized-tool acting as a guide but not a pure diagnostic tool. C4d's high specificity makes it valuable when resources are limited.

Contribution: This study validates the role of the Banff 2022 in our setting, aided by a computer-based tool, and demonstrates C4d's role as a practical DSA proxy, offering actionable solutions in resource-limited settings.

(239 words)

Key words: Renal transplantation, Banff 2022 classification, rejection biomarkers, digital pathology algorithm, C4d-DSA prediction

Background

Chronic kidney disease, affecting over five million South Africans(1), often progresses to end stage kidney disease (ESKD) where patients become dependent on renal replacement therapy (dialysis) or renal transplantation for survival(2-4). The definitive treatment for ESKD is renal transplantation which improves life expectancy and quality of life when compared to dialysis(5), and is thus a critical service in modern nephrology.

Although transplant success rates have significantly improved over the last seven decades, success is often hindered by immune-mediated rejection(6). An episode of acute rejection is a significant risk factor for graft loss, demonstrated in western populations(7, 8). Given this risk, urgent and accurate histopathologic diagnosis of rejection is essential to guide clinical decision-making.

The need for diagnostic guidelines for renal rejection gave rise to the Banff consensus meetings on allograft pathology, which started in 1991(9). These have been the cornerstone of rationalized, measurable histopathologic diagnoses in renal allograft pathology and rejection. Biennial iterations of the Banff criteria have provided an evolving framework for the histopathological diagnosis of renal allograft-associated pathology(9-11), which aid in clinical decision making.

The framework consists of semi-quantitative 'Banff Lesion Scores' based on the visualized histopathology of various compartments of renal tissue (glomeruli, tubules, interstitium and vasculature), enabling a standardized and comparable diagnostic approach(9). Initially based on expert opinion, the system is now heavily evidence-based, with large cohort studies and meta-analyses guiding changes(9). While a detailed discussion of these guidelines is beyond this article's scope, they are eloquently explained by the Banff Foundation's recent updates (10, 12).

The Banff consensus guidelines are updated every two years; an excellent summary of the year-on-year changes is given in a review article by Loupy *et al*(9). These iterations aim to improve the diagnostic sensitivity and specificity of guidelines, given the increasing dataset from which diagnostic criteria are decided. Each iteration hence has the potential to more accurately characterize and identify cases of renal rejection(9, 10).

Rejection is classified broadly as 'T-cell mediated rejection' (TCMR) and 'antibody mediated rejection' (AMR), both of which require urgent but different clinical management to ensure graft survival(13, 14).

TCMR is a cellular immune response to allograft human leukocyte antigen (HLA) molecules, which induces a directed cellular response against the transplanted kidney, manifesting histologically as interstitial inflammation, tubulitis, interstitial fibrosis and tubular atrophy(10, 12, 15). TCMR is categorized into acute and chronic active forms. In cases where there are insufficient criteria for a diagnosis of TCMR, and no intimal arteritis is present, a diagnosis of suspicious (borderline) for acute TCMR can be utilized(12). Ancillary studies are generally not needed in the diagnosis of TCMR.

TCMR remains the commonest form of rejection, having an overall prevalence of 11–30%, and 5–15% within the first year in international cohorts(7, 16). Importantly, evidence suggests that a single episode of TCMR places a recipient at risk of a future episode: nearly two thirds of those with an episode of TCMR had evidence of TCMR in future biopsies in a recent publication by Rampersad *et al*(7), which highlighted the importance of identification of this TCMR. A single-centre South African study showed a higher risk of TCMR, with a 37% risk of TCMR within the first year, possibly reflecting resource limitations within our setting(4).

AMR is an antibody-mediated attack of the transplant endothelium, specifically of the glomerular and tubular capillaries (so-called microvascular inflammation, MVI), and to a lesser extent medium- and large arteries(8, 17, 18). AMR was first recognized by Banff in 1997, with the first criteria for AMR defined in 2001(9). It is less studied, and seemingly less common, with overall prevalence from 1–21% in studies, most around 3–12%(19). Nevertheless, it is an important risk factor for graft loss. In our setting, Davidson *et al* showed an increased incidence of 34% within the first month, and 36% between 1 and 5 years, possibly reflecting the effects of resource limitation in our setting(4).

The alloimmune antibody response to the transplanted kidney is mediated by anti-HLA class I/II donor specific antibodies (DSA), targeting antigens on the donor organ endothelium(17, 20, 21). DSA are thus a strong predictor of AMR, and both pre-formed (pre-transplant) and *de novo* DSA are shown to play a role in the spectrum of AMR(21). The Banff 2022 guidelines continue to recommend DSA assessment for a diagnosis of AMR (with some nuances, discussed below). Detection of DSA around the time of biopsy is critical.

The laboratory measurement of DSA can be complex, with variability in available assays, difficulties with assay interferences, as well as the lack of standardized interpretation guidelines, discussed in depth by Haarberg *et al*(22). Furthermore, as is done in our setting, analysis is often batched due to the cost of the assay, delaying results and thus possibly delaying directed treatment of suspected rejection. Equipment breakdowns run the risk of halting DSA assessment, with obvious consequences for diagnosis of AMR.

The Banff consensus guidelines on renal rejection have long since recognised the role of DSA in rejection. Since 2001(23), Banff has also recognized the important role of the complement degradation product C4d as a histologic hallmark of AMR(24-27). C4d is formed as part of the classical complement pathway, activated by DSA during episodes of AMR. A by-product without fully understood function, C4d bonds covalently to endothelium, making it a robust, stable histologic marker, and is thus a pragmatic surrogate marker for DSA-mediated endothelial injury(28).

C4d is easily assessed on biopsy tissue, either through the more sensitive immunofluorescent techniques, or through standard immunohistochemistry(12); at our laboratory at Groote Schuur Hospital, immunohistochemistry is used. Given this relative ease of assessment, C4d status can be rendered at the same time as the histologic assessment of Banff lesion scores, significantly faster than serum assessment of DSA.

Thus, given the underlying biology of C4d making it a surrogate for DSA status, it can aid in identifying patients at high risk for AMR before DSA results are available and potentially decrease time to definitive clinical treatment. This has been demonstrated by several research groups(28-30), mostly in high-resource settings. To this end, the current Banff 2022 recognizes C4d in this light: C4d status in certain AMR categories can be substituted for DSA in providing molecular evidence of antibody mediated rejection(12, 24), which may expedite treatment in a resource-limited setting.

The evidence described by Davidson *et al*(4) from our hospital (spanning 2010–2015), which showed increased prevalence of both TCMR and AMR compared to international cohorts, highlights the importance of diagnosing these lesions accurately and timeously in our setting. While the HIV-positive to HIV-positive cohort has been recently studied(31), HIV-negative patients have not yet been studied for comparison since 2015 at our hospital.

Aims of this study

In this study we aimed to investigate the following:

(1) Characterize the landscape of renal allograft rejection and non-rejection pathologies within our hospital from 2015-2022: We described the renal transplant population based on historic biopsy reports at Groote Schuur Hospital from 2015–2022, and compare it to locally available data;

(2) Evaluate the diagnostic impact of Banff 2022 on historic diagnoses, using a computer-based tool: We applied the Banff 2022 guidelines to historic pathology reports to assess agreement between the new criteria and historic data, using a simple, automated Excel-based tool to identify cases;

(3) Assess C4d as a surrogate marker for DSA at our hospital: In our setting where DSA are not readily available, we assessed the performance of C4d immunohistochemistry as a predictor of DSA positivity.

Methods

Ethical approval

This study was approved by the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee (FHS-HREC 130/2022).

Study design, setting and population

This retrospective cohort study assessed renal transplant biopsy reports from patients at Groote Schuur Hospital for suspected rejection between September 2015 – December 2022. Patients were identified via Department of Nephrology biopsy records, and the National Health Laboratory Service (NHLS) TrakCare platform using matching SNOMED Clinical Terms(32). Any biopsied patient was included, except for HIV-positive patients who were excluded given the recent eloquent assessment of this cohort by Wearne *et al*(31). Our hospital does not perform protocol biopsies, and so all biopsies were for-cause. No slide review or human re-interpretation of the reported data was performed, in keeping with methods in similar studies in our setting(4, 31).

Data collection

Collected data from available histologic reports included demographics, all recorded Banff lesion scores, non-rejection pathology, and the final histologic diagnosis (referred to as the “reported diagnosis": recorded as positive, suspicious, or negative for rejection). At our laboratory, C4d immunohistochemistry is used (and not C4d immunofluorescence), and C4d scores were recorded.

Available serologic data (HIV status and DSA) were extracted for all patients. DSA testing done peri-biopsy (generally within 1 week before/after biopsy) was assessed to determine DSA status at the time of biopsy. DSA status was determined based on the reported DSA interpretation by NHLS Tissue Immunology laboratory at Groote Schuur Hospital. Briefly, this laboratory has access to confidential donor HLA profiles, and compare these to the recipient serum profiles to detect antibodies against the donor organ. Reports that had no explicit interpretation were excluded, as were DSA that were not done peri-biopsy, as defined above.

Data analysis

De-identified data were collated in Excel 2016 (Microsoft Office). Two time-cohorts were defined based on the Banff classification iteration used at our centre: 2015–2018 (which used Banff 2015) and 2019–2022 (which used Banff 2017). These cohorts were defined to reflect the Banff iterations that were generally used during these time periods. Additional variables were calculated as binary fields (namely C4d where C4d>0; MVI where MVI = $g + ptc > 0$) or as numerical fields (MVI score = $g + ptc$). The Banff '97 recommendations for adequacy(33) of ≥10 glomeruli and ≥2 arteries were not used as the number of arteries were too infrequently recorded (supplementary table 1). As such, the arterial requirement was dropped for adequacy and all biopsies with glomeruli were assessed.

The "calculated diagnoses" were determined using an Excel-based tool created by the authors, using conditional data retrieval and logic functions which implemented Banff 2022 criteria (link provided at end) The purpose was to assess whether the new criteria would impact rates of diagnosis (i.e. reclassify 'discordant' cases), while also acting as an audit of the laboratory's data capture practices; it was not a reflection or assessment of pathologist performance in historic reports. The agreement and discordance subsequently reported thus represent points of interrogation and assessment of the created tool.

Only the categories AMR, TCMR and suspicious (borderline) for acute TCMR (herewith "borderline rejection") were assessed. No retrospective calculations using Banff 2015 (used in 2015–2018) and Banff 2017 (used in 2019–2022) were performed. Biopsies lacking requisite Banff lesion scores were excluded from diagnostic calculations.

Data were analysed in RStudio (version 16.95.4 [25040241]) and IBM SPSS (version 30.0). Population descriptive statistics and temporal trends were assessed both at a biopsy and patient level using the Chi-square- and ANOVA-tests. Diagnostic agreement (reported vs. calculated diagnosis) was evaluated using Cohen's κ; cases that were reclassified by Banff 2022 (where the reported and calculated diagnoses were discordant) were tested with McNemar's test. Univariate logistic regression modelled various predictors (C4d, C4d Intensity (i.e. the Banff score), MVI, and the calculated MVI score) on the dichotomous outcome of DSA (positive/negative). Model fit used Nagelkerke R^2; classification accuracy was assessed at a 0.5 probability threshold. Significance was set at $p < 0.05$. Receiver operator curves (ROC) were generated to determine discriminative ability (Area under curve [AUC] with 95% CIs), with sensitivity and specificity calculated from the 2 2 tables. Only cases with all criteria for AMR including C4d and DSA were included in this assessment.

Graphical presentation was performed using RStudio (Version 2024.12.1+563) with R (version 4.2.2, R Foundation for Statistical Computing, Vienna, Austria). Bar charts comparing prevalence rates between time periods and sex groups were created using ggplot2 (v3.4.0). Timeline analysis was conducted using lubridate and ggplot2 packages. Set intersections and overlaps were visualized using UpSetR (v1.4.0).

Results

Demographics and biopsy characteristics

Case selection is summarized in figure 1**.** Initial screening identified 299 biopsies from the Department of Nephrology records. After exclusion of HIV-positive cases (n=84) and erroneously identified cases (n=18), 197 biopsies were available for retrospective review, from 135 unique patients.

There was a moderate male predominance in the cohort, with 80 males (59.3%) and 55 females (40.7%). The median age for the cohort was 38 years (IQR: 30 – 49 years). No significant difference in overall age of sexes was found. Eighty biopsies were done in females, and 117 biopsies in males.

After evaluating the total biopsies performed annually between 2015–2022, it was observed that only 4 biopsies were documented on the TrakCare system in 2015 (vs. an average of 27.6 biopsies/year for 2016–2022). This was because recording on the TrakCare system only began in September 2015 at our hospital; access to the previous laboratory information system to account for January–August 2015 was unavailable. Following the exclusion of 2015, there was a small but significant decline in the number of biopsies performed (R^2=0.765, p=0.01, ANOVA). When stratified by period, 124 biopsies (62.9%) were done between 2015–2018, and 73 (37.1%) between 2019–2022.

The mean patient age at biopsy decreased slightly but significantly (1.58-year reduction, p=0.005, ANOVA). This trend persisted when comparing the two time-cohorts, though with marginal significance (p=0.054). The sex distribution showed no significant variation throughout the study period.

Of the 135 patients biopsied, 46 patients (34.1%) underwent multiple biopsies, with multiple biopsy procedures accounting for 108 (54.8%) of the 197 biopsies, detailed in supplementary figure 1. Of note were two patients who each had four biopsies: patient 16 had one inadequate biopsy with subsequent repeat, and did not show any persistent/recurrence trends, over one year; patient 29 was diagnosed with TCMR, with subsequent persistent borderline rejection, all with acute tubular injury, over a period of four years.

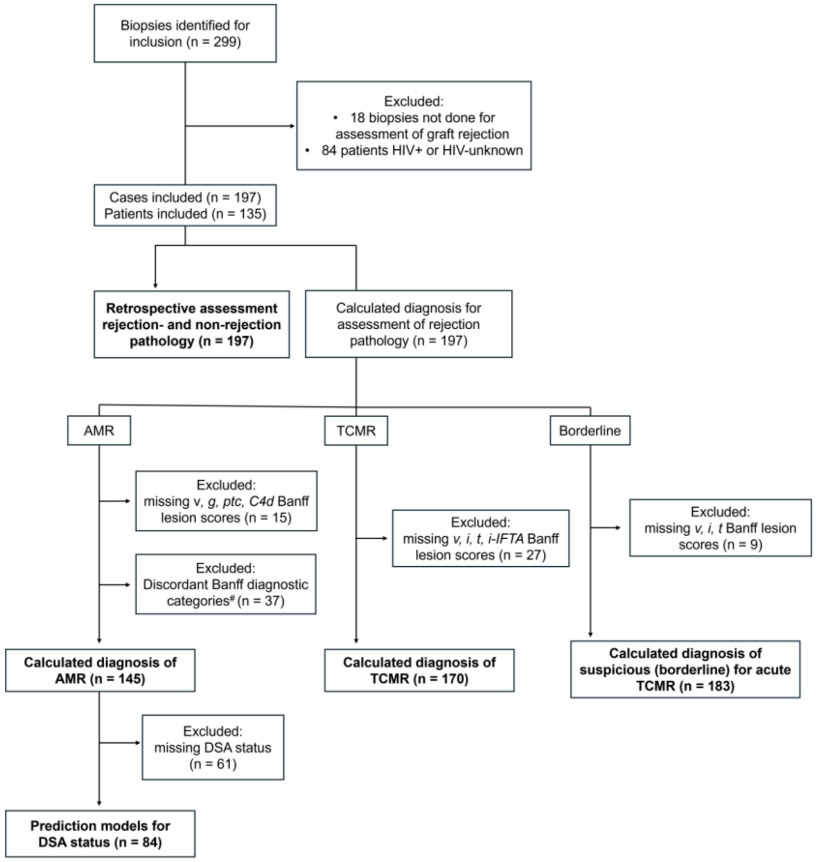
Biopsies identified for inclusion (n = 299)
Excluded:
• 18 biopsies not done for assessment of graft rejection
• 84 patients HIV+ or HIV-unknown
Cases included (n = 197)
Patients included (n = 135)
Retrospective assessment rejection- and non-rejection pathology (n = 197)
Calculated diagnosis for assessment of rejection pathology (n = 197)
AMR
TCMR
Borderline
Excluded: missing v, g, ptc, C4d Banff lesion scores (n = 15)
Excluded: Discordant Banff diagnostic categories# (n = 37)
Excluded: missing v, i, t, i-IFTA Banff lesion scores (n = 27)
Excluded: missing v, i, t Banff lesion scores (n = 9)
Calculated diagnosis of AMR (n = 145)
Calculated diagnosis of TCMR (n = 170)
Calculated diagnosis of suspicious (borderline) for acute TCMR (n = 183)
Excluded: missing DSA status (n = 61)
Prediction models for DSA status (n = 84)

Figure 1. Flow diagram of biopsies included in this study. Emboldened blocks represent the cohorts used for investigation. The review of reported (retrospective) diagnoses included 197 biopsies/cases from 135 unique patients. For the calculated diagnoses cases were excluded based on absence of requisite Banff lesion scores: AMR utilized 145 biopsies, TCMR utilized 170, and borderline rejection utilized 183 of the original 197 biopsies. For prediction modelling, only 84 of the 197 biopsies had complete data for inclusion. Italicized letters refer to Banff lesion scores, elaborated under 'abbreviations'.

- the excluded diagnostic categories included 'suspicious for ABMR' discontinued after Banff 2015, and the new categories 'probable AMR' and 'C4d staining with acute tubular injury' introduced in Banff 2022.

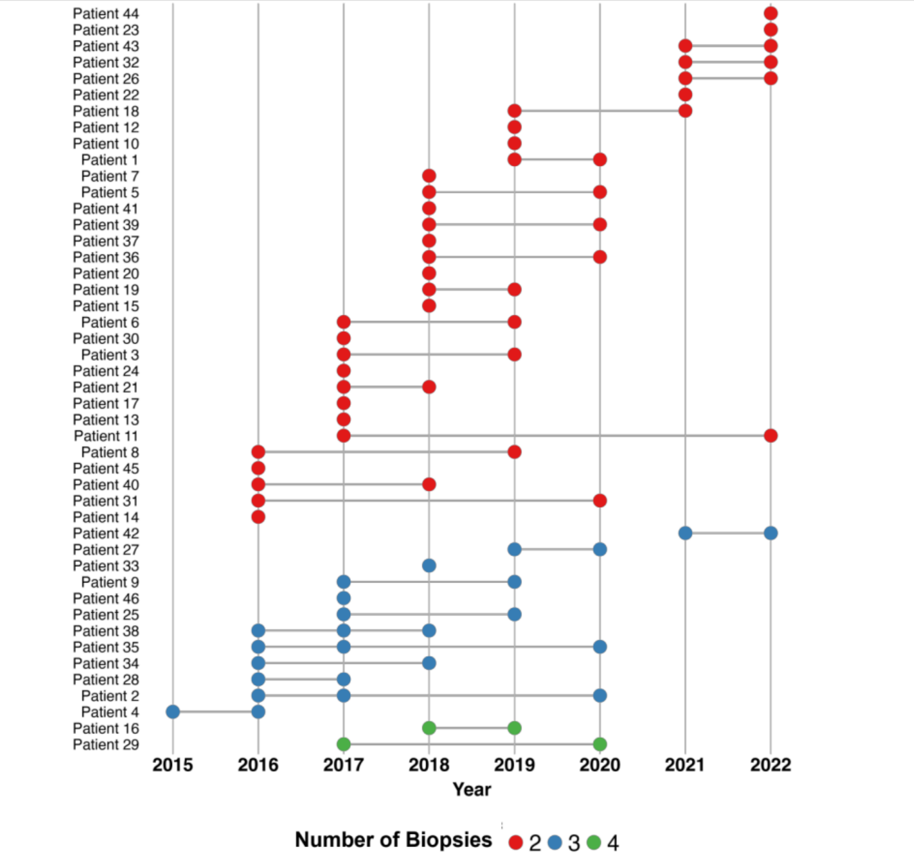

Figure 2. Biopsy timeline for patients with multiple procedures. A total of 46 patients (34.1%) had multiple for-cause biopsies, with 11 having three biopsies and 2 having four biopsies each. As our hospital does not do protocol biopsies, patients may not have many biopsies over the course of their transplant, as only symptomatic patients are targeted.

Non-rejection pathology prevalence

Non-rejection pathology is summarised in the UpSet plot in figure 3**.** These pathologies were commonly reported within the cohort, with 124 (62.9%) of the 197 biopsies having at least one non-rejection pathology. A substantial proportion of biopsies (n=66; 33.5%) had two or more non-rejection pathologies, with one patient having 3-, and one patient 4- diagnoses.

Acute tubular injury was the most common non-rejection pathology reported, seen in 92 (46.2%) of biopsies, co-occurring most commonly with features of pyelonephritis, drug-induced interstitial nephritis and calcineurin inhibitor toxicity. Calcineurin inhibitor toxicity in was seen in 28 (14.2%) of cases, co-occurring with multiple other non-rejection pathologies. Pyelonephritis was recorded in 19 cases (9.5%), and similarly co-occurred with multiple other diagnoses. Features of drug-induced interstitial nephritis were seen in 14 (7.1%) of cases. Two cases (1.0%) showed BK virus infection. The other less frequent non-rejection pathologies are reported in the figure.

Notably, in this cohort, no cases of CMV nephritis, reflux nephritis or post-transplant lymphoproliferative disorder were reported.

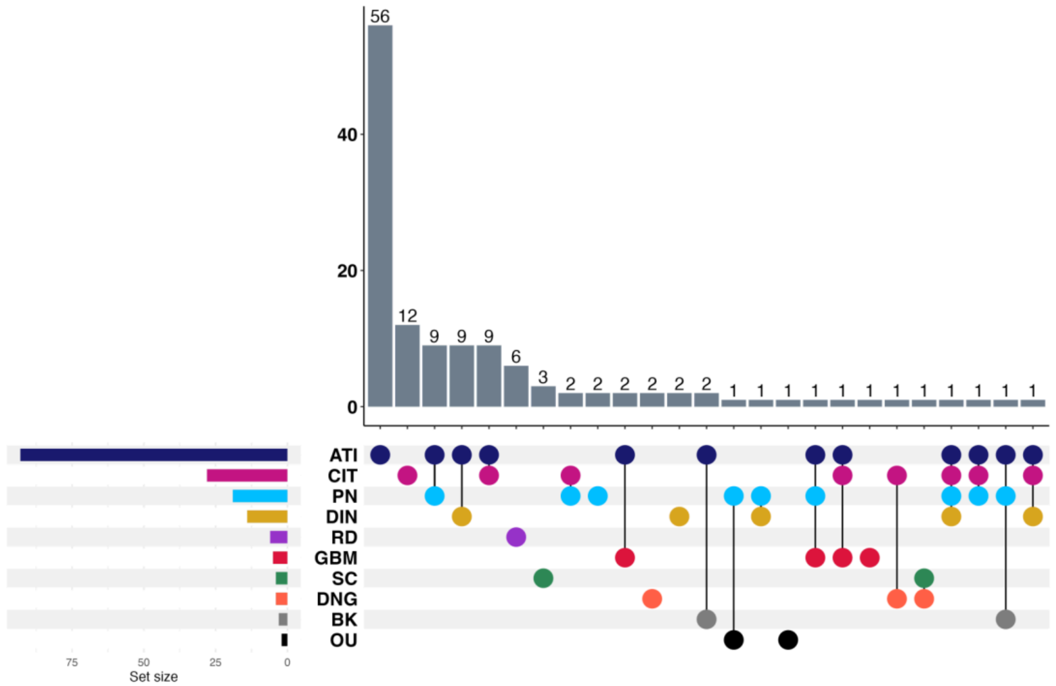

Figure 3. UpSet plot of non-rejection pathology. The set size (incidence of each of the pathologies identified) is shown on the lower left. The graph in the upper frame shows the incidence of individual- and co-occurring pathologies, with each column representing a unique pathology, or pathology conglomerate. No post-transplant lymphoproliferative disorders or CMV nephritis were identified.

ATI: *acute tubular injury;* ***CIT:*** *calcineurin-inhibitor toxicity;* ***PN:*** *pyelonephritis;* ***DIN:*** *drug-induced nephritis;* ***RD:*** *recurrent (primary) disease;*

GBM: *glomerular basement membrane changes;* ***SC:*** *subcapsular injury;* ***DNG:*** *de novo glomerulopathy;* ***BK:*** *BK polyomavirus infection;* ***OU:*** *obstructive uropathy*

Rejection prevalence during study period

A summary of rejection prevalence is provided in table 1 and figure 4. Of the 197 biopsies included, a diagnosis of AMR was reported in 15 cases (7.6%), with a diagnosis of 'suspicious for AMR' rendered in a further 39 cases (19.8%). A diagnosis of TCMR was seen in 34 cases (17.3%), with a diagnosis of borderline rejection seen in 29 cases (14.7%). Significant differences between time cohorts were noted for TCMR, with 28/34 cases (82.4%) occurring in 2015-2018 (p=0.010). No significant differences between time-cohorts were observed for AMR (positive or suspicious), or for borderline rejection. No proportional differences existed between sexes for any diagnoses (table 1b).

At an individual patient level (n=135), 30 patients (22.2%) had a diagnosis of TCMR, and 24 patients (17.8%) had at least one episode of borderline rejection. Fifteen patients (11.1%) had a diagnosis of AMR, and thirty-five (25.9%) of patients had at least one reported diagnosis of 'suspicious for AMR'.

With regards to repeated diagnosis of rejection, no patients had a repeat diagnosis of TCMR, but 5 patients (3.7%) had at least one co-occurrence of TCMR and borderline rejection. Similarly, no patients had a repeat diagnosis of AMR, however 4 patients (3.0%) having a co-occurrence of a 'positive for AMR' and 'suspicious for AMR'.

Table 1: Prevalence of rejection between 2015 – 2022

a. Prevalence by time cohort[#]

Diagnosis	Time period	Positive n (%)	Negative n (%)	p (Chi-square)
AMR (Positive)	Total	15 (7.6)	182 (92.4)	
	2015-2018	12 (9.7)	112 (90.3)	p=0.155
	2019-2022	3 (4.1)	70 (95.9)	
AMR (Suspicious)	Total	39 (19.8)	158 (80.2)	
	2015-2018	24 (19.4)	100 (80.6)	p=0.839
	2019-2022	15 (20.5)	58 (79.5)	
TCMR	Total	34 (17.3)	163 (82.7)	
	2015-2018	28 (22.6)	96 (77.4)	p=0.010
	2019-2022	6 (8.2)	67 (91.8)	
Borderline	Total	28 (14.2)	169 (85.8)	
	2015-2018	19 (15.3)	105 (84.7)	p=0.561
	2019-2022	9 (12.3)	64 (87.7)	

b. Prevalence by Sex[+]

Diagnosis	Sex	Positive n (%)	Negative n (%)	p (Chi-square)
AMR (Positive)	Total	15 (7.6)	182 (92.4)	
	Females	6 (7.5)	74 (92.5)	p=0.960
	Males	9 (7.7)	108 (92.3)	
AMR (Suspicious)	Total	39 (19.8)	158 (80.2)	
	Females	18 (22.5)	62 (77.5)	p=0.431
	Males	21 (17.9)	96 (82.1)	
TCMR	Total	34 (17.3)	163 (82.7)	
	Females	12 (15.0)	68 (85.0)	p=0.488
	Males	22 (18.8)	95 (81.2)	
Borderline	Total	28 (14.2)	169 (85.8)	
	Females	12 (15.0)	68 (85.0)	p=0.794
	Males	16 (13.7)	101 (86.3)	

***AMR**: antibody-mediated rejection; **TCMR**: T cell-mediated rejection;*
***Borderline**: Suspicious (borderline) for acute TCMR*

- n (2015-2018) = 124; n (2019-2022) = 73; n (Total) = 197
+ - n (Females) = 80; n (Males) = 117; n (Total) = 197

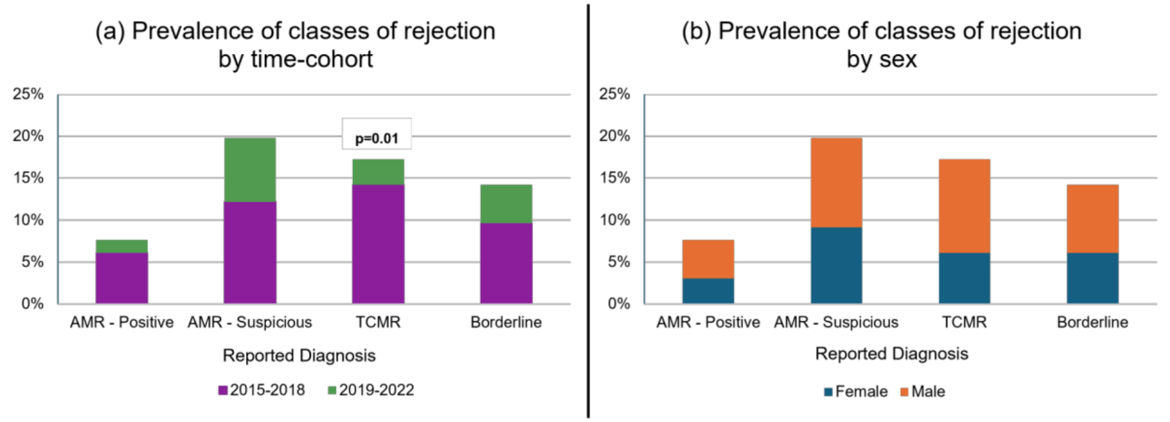

Figure 4. Prevalence of rejection over study period. (a) represents occurrence of rejection divided by time-cohort, with (b) showing division by sex. Only TCMR in (a) showed a significant difference between the two time-cohorts. There were no significant differences in occurrence of rejection type by sex.

AMR: *antibody-mediated rejection;* ***TCMR:*** *T-cell mediated rejection;* ***Borderline:*** *suspicious (borderline) for acute TCMR*

Agreement between reported and calculated diagnoses

The agreement between reported- and calculated diagnoses (using the Banff 2022 criteria) was evaluated for AMR, TCMR and Borderline rejection, using the excel-based tool that was developed by the authors. Table 2 provides a summary of the findings. For clarity, the reader is directed to the Banff Foundation's criteria for rejection(12).

Using the excel-based tool, AMR initially showed the lowest overall agreement of 72.9%. However, diagnostic sub-categories were not consistent over the study period and the Banff iterations used (supplementary table 2). Among the discordant cases, 30 cases were reported either positive or suspicious for AMR with no calculated diagnosis. Of these, the category of 'suspicious' predominated: 17 cases were diagnosed in the 2015-2018 cohort (where 'suspicious for ABMR' was an available diagnostic category by Banff 2015, but discontinued thereafter), and another 7 in the 2019-2022 cohort (where 'suspicious' was issued as a descriptive term/precautionary diagnosis and not an assertion of AMR, in the absence of all required features). Thus, only 6 cases called 'positive' were discordant, 5 of which were diagnosed in 2015-2018. Four were likely explained by insufficient *C4d* lesion scores, and two had co-existing acute TCMR, the higher thresholds for *g* and *ptc* lesion scores were not met.

Of the 17 cases of AMR that were calculated as positive but had no reported histologic diagnosis of AMR, 12 were accounted for by the new diagnostic categories 'probable AMR' and 'C4d staining with acute tubular injury', thus leaving only 5 cases (3 in 2015-2018, and 2 in 2019-2022) that were additionally identified through the calculated diagnosis (supplementary table 2). Review of these showed that of the 2 cases the tool labelled 'Active AMR', 1 had concurrent non-rejection inflammatory pathology, and 1 may have been additionally detected by the tool. Of the 3 cases labelled 'chronic (inactive) AMR' by the tool, 1 showed non-rejection inflammatory pathology, 1 showed recurrent disease, and 1 case (0.70%) may have been additionally detected by the tool.

Following the exclusion of these cases, 145 cases were included for assessment. AMR had an overall agreement of 92.41%, with good agreement between reported and calculated diagnoses ($\kappa=0.730$, $p<0.001$), which was slightly higher for the 2019-2022 group ($\kappa=0.803$, $p<0.001$) than the 2015-2018 group ($\kappa=0.681$, $p<0.001$). No significant differences were identified in distribution of the 11 discordant/reclassified cases overall, nor when stratified by time cohort.

Table 2: Agreement and discordance between reported and calculated diagnoses

a: Overall assessment of concordance

Diagnosis	Time period	Total Cases	Reported Positive	Calculated Positive	Concordance n (%)	Cohen's κ (p)
AMR	Total	145	25	24	134 (92.41)	0.730 (p<0.001)
	2015 - 2018	91	16	14	83 (91.21)	0.681 (p<0.001)
	2019 - 2022	54	9	10	51 (94.44)	0.809 (p<0.001)
TCMR	Total	170	29	36	157 (92.35)	0.753 (p<0.001)
	2015 - 2018	104	23	26	100 (96.15)	0.892 (p<0.001)
	2019 - 2022	66	6	11	57 (86.36)	0.400 (p>0.001)
Borderline	Total	183	27	46	151 (82.51)	0.455(p<0.001)
	2015 - 2018	117	18	29	99 (84.62)	0.519 (p<0.001)
	2019 - 2022	66	9	17	52 (78.79)	0.345 (p=0.003)

b: Breakdown of discordant (reclassified) results

Diagnosis	Time period	Total Cases	Reported+/ Calculated-	Reported-/ Calculated+	Discordance n (%)	p (McNemar's)
AMR	Total	145	6	5	11 (7.59)	p=1.000
	2015 - 2018	91	5	3	8 (8.79)	p=0.727
	2019 - 2022	54	1	2	3 (5.56)	p=1.000
TCMR	Total	170	3	10	13 (7.64)	p=0.092
	2015 - 2018	104	1	3	4 (3.85)	p=0.625
	2019 - 2022	66	2	7	9 (13.64)	p=0.180
Borderline	Total	183	7	25	32 (17.49)	p=0.002
	2015 - 2018	117	4	14	18 (15.38)	p<0.001
	2019 - 2022	66	3	11	14 (21.21)	p=0.057

Diagnostic sub-categories for of TCMR and borderline rejection were consistent over Banff iterations used during the study period.

TCMR showed fair to good agreement between reported and calculated diagnoses (κ=0.753, p<0.001), with an overall agreement of 92.35%. Concordance was significantly poorer for TCMR in 2019-2022 (κ=0.400, p<0.001). However, overall discordance was not significant between time-cohorts (p=0.092). Three cases were reported as TCMR but were not positive by calculation: 2 of these did however include descriptive terms suggesting TCMR, but were not outright in their diagnostic line; in the other case a transcription error was identified in the report. All 10 cases reported as negative but positive with the calculator could be explained: 5 cases showed features of AMR as the primary pathology, 2 showed disease recurrence, 2 showed evidence of a drug-induced nephritis, and 1 developed a de novo glomerulopathy.

Of interest was the large significant discordance in cases of borderline rejection. The overall agreement was 82.51% (κ= 0.445, p < 0.001). Discordance was 17.49%, with significant differences in discordant pairs (p=0.002), driven by 2015-2018 (p<0.001).

Seven cases were reported positive for borderline rejection but were not positive by calculation. Of these, 3 in 2015-2018 were indeed correct by Banff 2015 criteria (which changed in 2019), 2 used incorrect terminology (‘borderline rejection’ instead of ‘suspicious for ABMR’, a term available in Banff 2015 but thus incorrectly captured), 1 had a ‘*v*’ score that was in a comment explained to be due to recurrent primary disease (and so effectively regarded as ‘*v0*’, compatible with borderline rejection). One case (0.55%) did not meet Banff criteria for the period (Banff 2017) nor Banff 2022, and likely represents a false positive diagnosis.

Of the 25 cases reported negative but calculated positive, 17 could be explained by non-rejection pathology (5 showed acute tubular injury; 6 showed pyelonephritis; 3 showed calcineurin inhibitor toxicity; 1 showed drug induced nephritis; 1 showed disease recurrence; 1 BK virus infection was reported). There were 2 transcription errors, 1 scored in non-diagnostic scarred areas (and reported as such), and 1 biopsy that was reported as insufficient, but scores reported. There were thus 4 cases, 1 reported ‘unclear if rejection or secondary cause’, and 3 (1.64% of the 183 cases of borderline rejection) that appear to be additionally detected.

C4d as a predictor of DSA status

The outcomes of the four binary logistic regression models are summarized in supplementary table 3. These analyses revealed C4d was the most robust predictor of DSA status.

In these univariate analyses, binary C4d (positive/negative) showed an odds ratio (OR) of 7.97 (Confidence interval (CI): 1.67-38.01, p=0.009) with weak to moderate discrimination (area under curve (AUC)=0.62). This model showed high specificity (95.0%) but poor sensitivity (29.5%). C4d intensity showed similar results, with an OR of 4.99 (CI: 1.26-19.77, p=0.022), weak to moderate discrimination (AUC=0.627), with the same sensitivity, specificity, and overall accuracy (given calculation from the same 2 x 2 table values). The Nagelkerke R^2 showed that both C4d and C4d intensity had *a* very modest (values >0.1) but significant influence on DSA status.

Additionally investigated as a possible predictor of DSA status was microvascular inflammation. Neither the binary MVI nor the MVI Score models performed better than chance (AUC = 0.539 & 0.536), with non-significant p-values.

Discussion

This retrospective cohort study evaluated the prevalence of renal allograft rejection in HIV-negative patients at Groote Schuur Hospital and investigated diagnostic tools to aid in accurate and streamlined diagnoses.

Demographics and biopsy characteristics

In keeping with other South African cohorts from Cape Town(4, 31) and Johannesburg(34), our patients showed a male predominance, and was a younger population than international studies. This persistent sex disparity may reflect socioeconomic access inequalities, requiring further qualitative research.

The small but significant decrease in the number of biopsies is difficult to interpret in this study, given that our study lacks the HIV-positive population biopsied between 2015 and 2022, a group that comprised 28% of the originally identified population and may thus likely influenced trends. However other contributing factors may include changes in the area serviced (with diversion of work from the Southern Cape to other centers) between 2017 – 2021, as well as the COVID-19 pandemic where less biopsies were performed.

Comparison of rejection and non-rejection pathology with international cohorts

The overall prevalence of AMR, TCMR and borderline rejection in our study were consistent with international cohorts(7, 16, 19). As in other studies, TCMR was the most prevalent form of rejection noted in our HIV-negative cohort.

A comparison of our findings to two South African cohorts is shown in table 4. Despite relatively small sample sizes, all studies involved patients managed by the Department of Nephrology at Groote Schuur Hospital, minimizing confounders such as variations in treatment protocols and resource allocation.

Wearne *et al(31)* focused only HIV-positive patients – a group excluded from our analysis – but which remains comparable given the shared setting. When considering individual biopsies as cases, our study showed an increased prevalence of AMR and TCMR, likely because their protocol biopsies (none of which were reported to show rejection) likely diluted their reported prevalences. Since our cohort were not subjected to protocol biopsies, this dilution was absent. This confounder was helped when cases were defined by cumulative individual patient results, where their HIV-positive cohort showed increases in AMR and borderline rejection compared to our study, in keeping with the known higher rates in HIV-positive cohorts(35). The shorter follow-up (≤52 weeks) may explain the lack of similar trend in TCMR, and is an important factor that may under-estimate rejection in their study.

Table 4: Comparison of the present study to two other cohorts from our hospital

Study (ref.)	Biopsy level				Patient level			
	n	AMR n (%)	TCMR n (%)	Borderline n (%)	n	AMR n (%)	TCMR n (%)	Bo
Current study	197	15 (11)	30 (22)	24 (18)	35	15 (11)	30 (22)	2
Davidson *et al* (4)#	-	-	-	-	198	* (11–36)	* (18–37)	* (
Wearne *et al* (29)	179	9 (5)	12 (7)	25 (14)	50	9 (18)	9 (18)	1

***AMR**: antibody-mediated rejection; **TCMR**: T cell-mediated rejection; **Borderline**: Suspicious (borderline) for acut*

- *Davidson* et al *did not report overall prevalences, but rather prevalences at 1 month, 1 month – 1 year, and 1 y years. Reported are the minimum and maximum ranges for these time frames. Number of cases was not reportec*

Although Davidson *et al(4)* did not explicitly report overall prevalences for their study, our findings align with the lower end of their reported rejection ranges. Their study used Banff 2007 criteria, which possesses similar diagnostic criteria for rejection in our study cohort. This may suggest that differences in prevalence reflect improvements in patient care at our hospital. Future studies should incorporate clinical and histologic data to identify specific factors leading to this decline.

In terms of non-rejection related pathology, our cohort have a comparable overall prevalence compared to Kenyan(36) and Indian(37) cohorts. Of interest is our high rate of acute tubular injury, which is ~2–3x higher than the referenced cohorts. Although the nature of the biopsy in these groups is not stated (protocol vs. for-cause), this increased prevalence may speak to our hospitals for-cause biopsy strategy, where clinically ill patients are biopsied, with otherwise stable patients not biopsied; protocol biopsies in well patients with no co-existing pathology may act to dilute these rates. Calcineurin inhibitor toxicity in our cohort was similar to the Indian(37) and other western cohorts(38). Of interest was the absence of post-transplant lymphoproliferative disorder, and CMV nephritis in our cohort: our relatively small sample size likely explains their absence, given the rarity of these two entities. However, it is a good reminder for our practice to be aware of these entities, so as to not miss the rare presentation.

Agreement of the Banff 2022 criteria and utility of a computerized diagnostic tool

Comparing our cohorts reported histologic diagnoses to the diagnoses calculated by our simple Excel-based calculator provided a valuable retrospective audit, Banff iteration comparison, and a promising prospective role in our laboratory. The intentions behind an automated tool were to assess, firstly, if there were any significant changes to diagnoses when the Banff 2022 criteria were applied, and if so, what these changes were. Secondly, the tool was intended to check whether pathologists correctly translate parameters to arrive at the correct final diagnosis. The tool was created acknowledging the difficulties in use of the Banff framework, and not as a retrospective judgement.

In the case of AMR, the tool helped identify an important consideration when diagnosing AMR: the presence of TCMR upgrades the required Banff lesion scores to diagnose concurrent AMR. In 2 cases, this was not apparent from the report, and the tool helped identify both the reporting error and the likely correct diagnosis. It further acted to remind of the critical role of C4d staining in the diagnosis of AMR; apart from ‘chronic AMR’ and the new categories ‘Microvascular inflammation/injury (MVI), DSA-negative and C4d-negative’ and ‘Probable AMR’, AMR (moreover clinically actionable AMR) requires C4d staining to be present. In cases without C4d staining, a diagnosis of must be carefully re-assessed, highlighted by the 4 false-positives identified in our cohort.

On review of the discrepancies in TCMR in reported vs calculated cases, an important base truth was demonstrated: Banff lesion scores alone do not indicate rejection, and the clinical history, histology and scoring must be assessed as a whole. In the 10 discordant cases that were reported negative but calculated positive, it was noted that the tool was not discriminating cases with features of AMR or recurrent primary pathology (the latter as this was not often recorded in the diagnostic line). This is an important limitation identified, which may be addressed in future iterations of this tool.

Suspicious (borderline) for acute TCMR showed the lowest concordance. Although a small percentage were indeed due to Banff criteria changes (1.64%), one false positive was identified. An additional 25 cases (13.7%) were identified by the tool. From the available reported histologic data, it was clear that all but 3 cases (1.64%) had other co-existing non-rejection inflammatory processes, again underlying the importance of contextualizing the Banff lesion scores. This is an important limitation in our tool, and one with important clinical impact, as false positive results may lead to inappropriate treatment. Although the 2020 KDIGO guidelines recommend treating suspicious/borderline acute rejection (grade 1 evidence), the overall quality of the evidence is graded as "very low quality of evidence". In a resource-limited setting such as ours, it is sometime difficult to justify treatment where "the estimate of effect is uncertain, and often will be far from the truth"(13, 14). This further underscores the guiding, and not diagnostic, role that our tool plays.

The review also acted as an internal quality control, identifying 3 transcription errors and 2 cases where incorrect terminology was applied. In both cases, an incorrect diagnosis for the patient may result, underscoring the importance of case review. It is possible that these could have been avoided with the use of our diagnostic tool, which is designed to give the updated terminology based on the Banff 2022 criteria, and acting as a final guide for diagnostic lines.

Lastly, the tool demonstrated and reminded how complex and convoluted applying the Banff criteria can be. A category may have many inter-connected requirements and exceptions for each category, and occasionally categories are inter-dependent (active AMR is a good example of this(24)). Making a diagnosis is not only a challenge histologically, but even with accurately assessed histologic Banff lesion scores, compiling them into a diagnosis can be a confusing process. The tool, although not a true diagnostic calculator, aims to function as a prompt for the general pathologist to avoid misinterpretation or missed diagnostic considerations.

A beta model using a graphics user interface asking for additional confounders is in development at the time of submission and will hopefully further contribute to diagnostic guidance.

Predictors of DSA positivity in our setting

C4d has been emphasized by the Banff 2022 framework as a vital component for the diagnosis of AMR. The recent update highlights that when C4d is positive, even where MVI thresholds are not met, AMR can be diagnosed (reference (24); see reproduction in supplementary figure 1). Given the role of C4d as a surrogate marker for DSA and considering the difficulties in assessing DSA in our setting, evaluating the performance of C4d at our centre is critically needed. This was additionally important to aid clinical colleagues in understanding the critical role of this marker and decrease sole reliance on DSA, given that other robust diagnostic molecular markers are still under investigation.

The univariate modelling of four predictive factors demonstrated that binary C4d status and numeric C4d intensity, although modest in power, were in line with the known role of C4d as a surrogate for DSA status(28, 29, 39). Microvascular inflammation showed no predictive power in our cohort.

The high specificity of C4d immunohistochemistry in detecting DSA negative cases at our hospital may prove a useful cost-saving tool. Given the difficulties with DSA, one of which is the limited volume that can be processed, using C4d to guide decision making decreases the pressure placed on the DSA service. A small proportion (5%) of cases may show false C4d positivity (for example, in ABO-incompatible transplants(28)). However given that the managing nephrologists know the transplant type, and given their clinical suspicion and the other histologic features, these considerations would, in this 5%, inform the decision to test DSA; C4d status and intensity becomes a suggestive tool, not a rule in/rule out beacon.

Of some concern is the poor sensitivity of our C4d immunohistochemistry at our hospital, which would miss ~70% of true DSA-positive cases. This may be affected by many factors in the chain of biopsy handling, such as variation in tissue fixation times, issues in tissue processing, as well as the immunohistochemical protocol used by our laboratory. These assumptions are supported by the significant (>0.1) but low Nagelkerke R^2, emphasizing that the variance seen is influenced by factors not identified by our study, which require further investigation.

One contributing factor may be the known poorer sensitivity and specificity of C4d immunohistochemistry (used at our hospital), versus C4d immunofluorescence(28, 30, 39). Immunofluorescence has also been shown to have a stronger agreement between pathologists(39), and is a more robust means of C4d assessment. It does, however, have technical requirements (additional tissue examined at the time of biopsy by dissecting microscope, specialized storage media, additional staining techniques, etc). It is worthy of future research to investigate this modality in our setting, as a more accurate C4d identification may aid to decreased long-term costs and faster patient intervention, with hopefully decreased graft failure and better patient outcomes.

Limitations

One of the primary limitations in this study was the lack of sufficient clinical information. Although age and sex are obtainable from the laboratory information system, more detailed clinical histories are often not available on request forms. Furthermore, the aetiology of the antecedent ESKD was largely unknown, which may have import for long term graft survival, given varying risks of recurrent disease. Knowledge of this and factors such as ischaemic time may help to understand, for example, the high rate of ATI seen in this study.

In addition, our study did not know the type of donor/allograft (e.g. cadaveric donor, living relative donor, donation after brainstem death, etc). Previous studies have shown that these have an effect on long term graft survival in our setting(4), and replicating this with a later cohort may give valuable reflective clinical insights for the clinical nephrology team.

Our computerized tool had limitations: it was not built to account for the non-specificity of some Banff lesion scores, which can occur due to non-rejection pathology. Thus, future version will need more user input to try and account for this.

Lastly, our cohort is relatively small, with an even smaller portion available for predictive modelling. Future research focusing on larger cohorts, and perhaps collaboration with other local and sub-Saharan centres, would give more robust statistics for C4d as a predictor of DSA status, an invaluable tool in a resource limited setting.

Conclusion

This study provides insights into the diagnostic landscape of renal allograft rejection in a South African cohort. The study further demonstrates the utility of the Banff 2022 criteria, and the role of a computer-based diagnostic tool in a resource-limited setting. The prevalence of various types of rejection appears stable when compared to previous cohorts in our setting. While our rates of rejection- and non-rejection pathology appear high, these are likely inflated due to the for-cause biopsy practice (and not protocol biopsy) at our hospital.

Our analysis demonstrates that while the updated Banff framework offers some improvement in diagnostic standardization. Although not acting as a pure diagnostic tool, the tool could act as an important adjunct for guidance of the general pathologist, and ultimately helped identify possible additional cases, a minority representing potential new diagnoses. This underscores that such tools serve best as adjuncts to, rather than replacements for, expert pathological assessment.

C4d immunohistochemistry emerged as a valuable, albeit imperfect, surrogate for DSA status, with high specificity but limited sensitivity. This underscores its potential to guide early therapeutic decisions when DSA results are pending, though its clinical application must account for false negatives. Future studies should explore whether transitioning to C4d immunofluorescence could enhance detection rates, given its superior performance in other populations.

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