Composition of the neutralising antibody response predicts risk of BK virus DNAaemia in recipients of kidney transplants

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Summary

Background BK polyomavirus (BKV) DNAaemia occurs in 10% of recipients of kidney transplants, contributing to premature allograft failure. Evidence suggests disease is donor derived. Hypothetically, recipient infection with a different BKV serotype increases risk due to poorer immunological control. Thus, understanding the composition and activity of the humoral anti-BKV responses in donor/recipient (D/R) pairs is critical.

Methods Using 224 paired pre-transplant D/R samples, BKV VP1 genotype-specific pseudoviruses were employed to define the breadth of the antibody response against different serotypes (ELISA) and, to characterise specific neutralising activity (nAb) using the 50% inhibitory concentration (LogIC50). Mismatch (MM) ratios were calculated using the ratio of recipient ELISA or nAb reactive BKV serotypes relative to the number of donor reactive serotypes.

Findings BKV DNAaemia was observed in 28/224 recipients of kidney transplants. These recipients had lower nAb titres against all the serotypes, with median logIC50 values of 1.19–2.91, compared to non-viraemic recipients' median logIC50 values of 2.13–3.30. nAb D/R MM ratios >0.67 associated with significantly higher risk of BKV viraemia, with an adjusted odds ratio of 5.12 (95% CI 2.07 to 13.04; $p < 0.001$). Notably, a mismatch against donor serotype Ic and II associated with adjusted odds ratios of 8.12 (95% CI 2.10 to 35.61; $p = 0.002$) and 4.52 (95% CI 1.19 to 19.23; $p = 0.03$) respectively. 21 recipients demonstrated broadly neutralising responses against all the serotypes, none of whom developed BKV DNAaemia post-transplant. In contrast, there was poor concordance with PsV-specific ELISA data that quantified the total antibody response against different serotypes.

Interpretation BKV nAb mismatch predicts post-transplant BKV DNAaemia. Specific mismatches in nAb, rather than total seroreactivity, are key indicators of BKV risk post-transplant. This has the potential to risk-stratify individuals and improve clinical outcomes by influencing the frequency of monitoring and individualised tailoring of immunosuppression. Furthermore, detailed examination of individuals with broadly neutralising responses may provide future therapeutic strategies.

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Keywords: Post-transplant infections; BK virus; Kidney transplantation; BKV serotyping

Introduction

BK polyomavirus (BKV) establishes asymptomatic lifelong infections of the host, with an estimated seroprevalence >75% in the immunocompetent population.[1,2] Active BKV infection following kidney transplantation is a major cause of early graft failure in

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Research in context

Evidence before this study

Accumulating evidence suggests that BKV donor genotype and recipient antibody mismatches contribute to the risk phenotype post-transplant. However, due to limited participant numbers in previous studies (generally <120 transplant pairs), coupled with a failure of some studies to detect BKV-II or III, it has not been possible to investigate the impact of all potential specific neutralising antibody mismatches thoroughly.

Added value of this study

This study investigates the use of BKV serotype mismatches in predicting post-transplant DNAaemia. We have conducted a clinical and immunological study of sera taken from 224 paired donors and recipients of kidney transplants, making it one of the largest studies in this area. We have employed pseudotype BKV viruses to systematically characterise the identity and neutralising activity of the humoral immune response in both donors and recipients and used this to stratify risk for BKV viraemia. Specifically, we show that although mismatches in the humoral immune response poorly predicted risk, specific evaluation of the neutralising antibody response demonstrated that D/R mismatches could be used to score risk of, and be used to predict the onset of, post-transplant disease—suggesting that the neutralising antibody component of the total humoral response is likely important for control of disease. Consistent with this, we observe that a subset of individuals with broadly neutralising antibodies against all genotypes of BKV are protected from post-transplant BKV viraemia.

Implications of all the available evidence

Our findings support the hypothesis that donor and recipient BKV serostatus mismatch is a risk factor for disease; and, that mismatch ratios, as well as specific mismatches, can be used to predict risk. These data demonstrate the importance of neutralising antibody responses in control of BKV pathogenesis in identifying high risk situations, revealed by immunological mismatches between donor and recipient, and suggest that a subset of individuals can make broadly neutralising antibody responses. Thus, these data have the potential to have both immediate and long-term impact on BKV disease through better clinical management and informing future development of novel antibody-based therapeutics, respectively.

approximately half of those affected.[3] Whether recipient BKV is due to primary infection, whether BKV-naïve or a previously unencountered viral genotype, or represents recipient-derived re-activation of latent virus, remains unclear.

The 4 subgroups of BKV genotypes (Ia, Ib1, Ib2, Ic, II, III, IVb1 and, IVc2) represent 5 distinct serotypes, since BKV-Ib1 and BKV-Ib2 have been shown to have discernibly unique cellular interactions, along with serotypes II, III and IV.[4] However, BKV-Ia and BKV-Ib1 vary genetically in sequences outside of the VP1 gene and therefore produce functionally identical experimental pseudoviruses. Genotype I is most prevalent worldwide, although there is variation even on a continental level with Ib2 most prevalent in the UK[5,6] and USA,[7] Ib1 in the Netherlands[8] and Poland and Ic in Germany,[9] whilst Ia is most prevalent in Africa, Ib1 in southeast Asia, Ic in Japan[6] and IV in China and other parts of east Asia but rare in Africa.[10–12] BKV subtypes II and III occur at much lower frequencies and are completely absent in some studies in Japan, Poland and Argentina.[11,13–15]

There is a growing body of evidence that donor-derived infection may be an important factor in post-transplant infection.[16–18] However, pre-transplant seropositivity to BKV has not been shown to protect against viraemia,[19] suggesting that it is not the presence or absence of antibodies that determines disease, but instead, the specificity of the response may be more important. Dakroub et al. investigated this in more detail by using a VLP-based ELISA system to examine anti-BKV serotype I and IV antibodies.[20] They showed that the probability of BKV DNAaemia in recipients seronegative for anti-BKV-I with donors who are seropositive (D + R-) was 37.5%, compared to 4.3% in D-R+. Furthermore, they found that lower recipient antibody titres were associated with a higher proportion of presumptive BKV nephropathy (BKVN) of 50%, and that recipients seronegative for BKV-IV were at highest risk of BKV-I DNAaemia of 25.5%, compared to 2.3% with BKV-II or BKV-IV DNAaemia, suggesting that not all genotypes may confer similar risk. Abend et al. combined both an ELISA and neutralising antibody (nAb) approach and found no correlation between ELISA serostatus and post-transplant DNAaemia, but nAb serostatus was strongly predictive.[21] Indeed, a strong positive correlation between donor BKV nAb seroreactivity and incidence of recipient BKV DNAaemia and nephropathy has been demonstrated in live donor KT pairs.[16,22] It is unclear if this observation reflects that higher seroreactivity is indicative of a higher BKV viral load or reservoir within the donated kidney, or suggests transmission of a more virulent, or previously unencountered viral genotype. Similarly, recipients with poor nAb responses against BKV are at increased risk of BKV disease, and conversely, high nAb titres (>4 $\log_{10}$) against the same serotype of BKV as that of their donor conferred significant protection against BKV disease as shown by Solis et al.[23] Several studies have attempted to look at the nAb serostatus against different serotypes

but have very low or absent numbers of BKV-II, III and IV,[21,23] despite evidence that all genotypes can be found in biopsies of kidneys with BKVN.[24]

Several serological parameters are likely important for the control of BKV virus post-transplant, including serotype mismatches between donor and recipient: recipient BKV immune status, quality of the antibody response, as well as the clear geographical distribution of BKV serotypes–indicating the potential importance of host ethnicity in the transplant setting. To address these concepts, we conducted a large cohort study of 224 kT pairs in a multi-ethnic transplant population to characterise the relationship between donor and recipient humoral responses to BKV serotypes and to predict the risk of post-transplant infection. In doing so, we now identify that high-risk individuals can be identified by the degree and specificity of nAb mismatch compared with donor serotypes.

Methods

Study design and population

Adult recipients of kidney transplants (KTs) were recruited from the Royal Free Hospital, London. Pre-transplant deceased donor serum samples were predominantly obtained from the Quality in Organ Donation (QuOD) bioresource, a National Health Service Blood and Transplant (NHSBT)-funded project, that collects donor samples peri-donation. Each donor can be traced to the implanting centre, facilitating identification of corresponding recipients. All QuOD donors with kidneys transplanted in the Royal Free Hospital, since the biobank was established in 2014, were identified and paired recipients were recruited into the study. Pre-transplant recipient sera were obtained from the Anthony Nolan laboratories, solid organ group or prospectively collected as plasma (including live donors pairs recruited between June 2019 and June 2020) prior to the introduction of immunosuppression. All live donor transplant pairs, or recipients over 18 years old with available pre-transplant donor and recipient samples, were approached for recruitment into the study.

ELISA

Donor and recipient samples were examined for anti-BKV antibodies using a commercial kit (ELISA-VIDITEST anti-BKV IgG, Vidia, Czech Republic), which utilises species-specific recombinant antigens in the form of VP1 viral-like particles (VLP) derived from the BKV-I and BKV-IV dominant serotypes.[25] Based on whole BKV IgG serostatus, donor/recipient (D/R) pairs were divided into cohorts based on donor (D) and recipient (R) seropositive (+) or seronegative (–) status.

Pseudovirus (PsV) production

Pseudovirions were produced using plasmids expressing the major capsid protein VP1 of BKV genotypes Ia, Ib2, Ic, II, III, IVb1 and IVc2, with VP2, VP3 and a reporter plasmid encoding Gaussia luciferase, as described in other studies[4,23,26] (gifted by Dr Chris Buck, NIH, Maryland, USA).

Neutralisation assays (NA)

Heat inactivated (56 °C for 30 min) plasma/serum samples were tested using a 1:5 serial dilution, followed by the addition of a standardised volume of each serotype PsV in triplicate. The sample dilutions, positive control (PsV only) or negative control were pre-incubated in DMEM (Lonza, cat: 12–604 F) with 10% foetal bovine serum (Gibco, cat: A5256701) and 1% penicillin-streptomycin (Gibco, cat: 15140122) for 1 h at 37 °C then further incubated at 37 °C for 72 h with HEK293TT cells (ATCC: CRL-3467, RRID: CVCL_1D85, gifted by Dr. Andrew Macdonald, University of Leeds). This cell line has been validated by ATCC and 3 monthly mycoplasma testing was performed at the time of the study.

Neutralisation was calculated using the proportion of luminometric Gaussia luciferase (Thermo scientific, Pierce, Cat: 16161) signal reduction of each sample dilution relative to the maximum signal using 6 replicates of the positive control. This was used to determine the sample dilution required to achieve 50% inhibition of PsV infectivity, expressed as $\log_{10}$ of the IC50 (LogIC50). Individuals were considered non-neutralising against a particular serotype if the logIC50 was below a cut off, defined by the average LogIC50, plus 2 standard deviations, of 58 individuals who were seronegative on ELISA kit testing.

PsV-specific ELISAs

96 well PolySorp plates (Nunc™) were coated overnight with 1 μg/ml of one PsV per plate in coating buffer (pH 9.4–9.8). After washing (PBS-Tween 0.05%; PBS-T; PBS pH 7.4 Gibco, cat: 10010023 and tween 20 Sigma–Aldrich, cat P1379) and blocking (2% bovine serum albumin (BSA; Sigma–Aldrich, cat 05470)/PBS-T) for 1 h, samples were added to the plates in duplicate at dilutions of 1:50 and 1:500 for each PsV and incubated for 1 h. After washing, peroxidase-conjugated antibody (goat anti-human IgG, Jackson ImmunoResearch Labs, cat: 109-035-170, RRID:AB_2810887) was added and incubated for 1 h. A final 3 washes were performed prior to adding tetramethylbenzidine peroxidase substrate (TMB; Thermo Scientific, cat: 34029) for 10 min, followed by 1 M phosphoric acid to stop the reaction. Plates were read immediately at an optical density of 450 nm using an Emax microplate reader. Seronegativity was defined using the average OD value of true negatives (negative by ELISA kit and with no neutralising activity) plus 2 standard deviations. To compare different PsVs and individuals across different plates, individual OD values are presented relative to the average of the positive control (PC) of each plate.

Data collection and analysis

Recipient data were collected from clinical databases regarding demographics, co-morbidities, presence of BKV DNAaemia and corresponding characteristics (peak viral load, time to onset and BKVN), as well as donor demographics. There was a minimum follow-up BKV DNA screening of 12 months, and ad-hoc testing up to 60 months. Sex and ethnicity were self-reported. KT pairs were categorised into significant DNAaemia of >1000 copies/ml, or the non-viraemic populations. The latter included those with low level DNAaemia of <1000 copies/ml occurring within 12 months post-transplant and late DNAaemia of >1000 copies/ml occurring after 12 months post-transplant.

Recipient mismatch (MM) was defined as the absence of evidence of antibodies detected by ELISA (ELISA MM) or neutralisation (NA MM) against the corresponding BKV serotype detected in the donor sera. A mismatch ratio for each pair was calculated using the number of recipient MM to the donor profile, relative to the total number of serotypes exposed to from the donor.

Statistics

Baseline characteristics of the study population, stratified by BKV DNAaemia were compared using Chi-Square for categorical variables, and Kruskal–Wallis tests or ANOVA for continuous variables, as appropriate. Firth logistic regression was used to describe the association between NA MM ratio and BKV DNAaemia status. It was decided *a priori* to include age and sex in all models; models for the primary outcome were additionally adjusted for recipient ethnicity and acute rejection within 12 months of transplant. We also analysed the association between NA MM ratio and the following secondary outcomes: transplant type, BMI, HLA mismatches, pre-emptive transplants, corticosteroid use, CMV DNAaemia, and donor age, sex and ethnicity. Individual serotype mismatches were additionally adjusted for all the other serotype mismatches. Logistic regression statistical analyses were done using R (version 4.0.2, R Foundation for Statistical Computing, Vienna, Austria). ROC analyses of PsV-specific ELISAs and nAb mismatch was performed using GraphPad Prism software (GraphPad Software V10.0.2, La Jolla, USA). Data was considered significant where p-values were <0.05.

Ethics

The study ethics approvals were provided by the NHS (NRES REC 21/WA/0388) and University College London institutional ethical committee (B-ERC ref: NC.2018.010). Written informed consent was obtained for each participant.

Role of funders

The funders had no role in study design, data collection, analyses, interpretation, or writing of the report.

Results

Study cohort

Between July 2014 and October 2021, 224 kidney transplant pairs were recruited; 21 live donor pairs and 203 were deceased donor pairs. All recipients were given standard induction immunosuppression with Basiliximab and intravenous methylprednisolone and, in most cases, maintenance immunosuppression using tacrolimus and mycophenolate mofetil, with early steroid withdrawal. 6 pairs were excluded from D/R comparison due to early graft loss or absence of follow-up data. Screening for blood BKV DNA was performed 2-monthly for the first-year post-transplant and based on clinical need thereafter, and immunosuppression was reduced when BKV DNA exceeded 2000 copies/ml by reducing/withdrawing the anti-proliferative agent (and steroids), followed by a reduction in calcineurin inhibitor dose. 45 recipients developed post-transplant BKV DNAaemia, of which 28 recipients developed significant DNAaemia >1000 copies/ml within 12 months post-transplant. Those with low level DNAaemia <1000 copies/mL (n = 13) and late DNAaemia >12 months post-transplant (n = 4) were included in the non-viraemic population as described (n = 190). Due to insufficient available sample, only 212 kT pairs were available for PsV-specific ELISA analyses. The cohort demographics stratified by BKV DNAaemia or no BKV DNAaemia are described in Table 1. Participants had a mean age of 52 (SD 13.7) years and 40% were female. A majority (89%) was on their first kidney graft, and 71% of them had ≤3 HLA mismatches. Participants with BKV DNAaemia experienced more rejection in their first-year post-transplant (21.4% vs. 7.4%).

D/R serostatus cohorts confer different risks of BKV DNAaemia

To assess whether BKV serostatus was an important predictor for DNAaemia in our cohort, BKV serostatus was determined in a preliminary study of 175 adult D/R pairs using a commercially available ELISA kit. Of these, 125 pairs were reported D + R+, 18 were D + R-, 25 were D-R+ and 7 pairs were D-R- (Fig. 1a). In these cohorts, the highest rate of post-transplant BKV DNAaemia was in the D + R-cohort (n = 8; 44%) followed by D + R+ (n = 23; 18.4%), D-R- (n = 1; 14.3%) and finally, D-R+ (n = 2; 8%). This difference was statistically significant (Chi-square; Fig. 1b). However, there was limited clinical utility as a risk prediction tool as it did not identify risk in the D+R+ cohort where most pairs were found. The positive predictive value of a seropositive donor was 21.68% (CI 19.49–24.04).

PsV-specific ELISA mismatch weakly predicts BKV DNAaemia

To further understand the importance of differences in the donor and recipient immunity we developed and utilised PsV-specific ELISAs to investigate the specific

	No BKV DNAaemia (n = 190)	BKV DNAaemia (n = 28)	Overall (n = 218)	p-value
Recipient characteristics				
Recipient age	52.6 (13.2)	48.5 (16.6)	52.0 (13.7)	0.20
Sex				
Female	81 (42.6)	7 (25.0)	88 (40.4)	0.21
Male	109 (57.4)	21 (75.0)	130 (59.6)	
Ethnicity				
White	70 (36.8)	9 (32.1)	79 (36.2)	0.52
Non-white	94 (49.5)	18 (64.3)	112 (51.4)	
BMI				
BMI ≤ 25	73 (38.4)	14 (50.0)	87 (39.9)	0.52
BMI > 25	116 (61.1)	14 (50.0)	130 (59.6)	
Donor characteristics				
Age	51.6 (14.2)	48.2 (17.1)	51.1 (14.6)	0.30
Sex				
Female	78 (41.1)	8 (28.6)	86 (39.4)	0.53
Male	112 (58.9)	19 (67.9)	131 (60.1)	
Ethnicity				
White	171 (90.0)	23 (82.1)	194 (89.0)	0.75
Non-white	19 (10.0)	4 (14.3)	23 (10.6)	
Transplant type				
Live	18 (9.5)	3 (10.7)	21 (9.6)	1
DBD	120 (63.2)	18 (64.3)	138 (63.3)	
DCD	52 (27.4)	7 (25.0)	59 (27.1)	
HLA mismatch				
≤3	134 (70.5)	21 (75.0)	155 (71.1)	0.89
>3	56 (29.5)	7 (25.0)	63 (28.9)	
Graft number				
1st	167 (87.9)	26 (92.9)	193 (88.5)	0.93
2nd	19 (10.0)	2 (7.1)	21 (9.6)	
3rd	4 (2.1)	0 (0)	4 (1.8)	
Rejection in 1st year	14 (7.4)	6 (21.4)	20 (9.2)	0.05
CMV DNAaemia >2000 copies/mL	60 (31.6)	11 (39.3)	71 (32.6)	0.73
Corticosteroid use	80 (42.1)	15 (53.6)	95 (43.6)	0.54
Basiliximab induction	190 (100)	28 (100)	218 (100)	1
Cold ischaemic time	11 h 18 min (4 h 48 min)	9 h 49 min (4 h 29 min)	11 h 06 min (4 h 47 min)	0.12
CMV serostatus				
+/+	68 (35.8)	8 (28.6)	76 (34.9)	0.80
+/−	18 (9.5)	6 (21.4)	24 (11.0)	
−/+	78 (41.1)	11 (39.3)	89 (40.8)	
−/−	23 (12.1)	2 (7.1)	25 (11.5)	

Table 1: **Demographics of BKV viraemic and non-viraemic control cohorts (p-values determined using Chi-Square and Kruskal–Wallis tests or ANOVA where appropriate), represented as mean (SD) for continuous variables or n (%) for categorical variables.**

composition of the BKV humoral immune response. D/R serological status to all 7 specific BKV serotypes were determined using PsV-specific ELISAs in 212 kT pairs (comprising the original 175 analysed in the preliminary data set and an additional 37 collected during the course of the study) and then analysed according to BKV outcome post-transplant. The mismatch (MM) score for each pair was calculated and correlated with presence or absence of DNAaemia. The median MM score in the viraemic cohort was 0.79 (IQR 0–1) and 0 (IQR 0–0.62; $p < 0.001$) in the non-viraemic population. Using ROC curve analysis, a higher ELISA MM was associated with BKV DNAaemia, and a MM ratio cut off >0.69, produced a likelihood ratio of BKV disease of 2.78 ($p = 0.0011$) with a sensitivity 53.85% (95% CI 35.46–71.24%) and specificity 80.65% (CI

a

# pairs		RECIPIENT	
		Seropositive	Seronegative
DONOR	Seropositive	125	18
	Seronegative	25	7

b

% with BK viraemia		RECIPIENT	
		Seropositive	Seronegative
DONOR	Seropositive	18.4	44.4
	Seronegative	8.0	14.3

Chi-square p=0.02

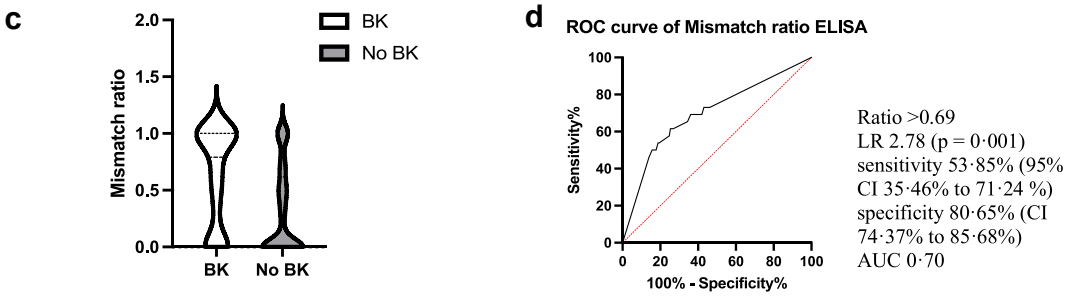

Fig. 1: **Distribution of D/R pairs in cohorts defined by D/R serostatus using a BKV IgG kit and the association post-transplant BKV DNAaemia with D/R serostatus groups and PsV-specific ELISA mismatch**. (a) Number of donor–recipient pairs in each D/R cohort defined by anti-BKV IgG commercial ELISA assay. (b) Association between BKV D/R seropositivity status and the development of BKV DNAaemia post-transplant showing highest rates of BKV DNAaemia seen in D + R- (44%), followed by D + R+ (18.4%). (c) PsV-specific ELISA mismatch ratios and BKV DNAaemia for all kidney transplant pairs. Violin plot showing the ELISA MM ratios for the total viraemic and non-viraemic cohorts using PsV-specific ELISA assays and (d) ROC curve analysis showing that ELISA mismatch ratios weakly predicts post-transplant BKV DNAaemia, with an AUC < 0.7 and a ROC generated p-value of 0.001, in the whole cohort of 212 recipients, regardless of D/R BKV serostatus.

74.37–85.68%) but with an AUC of 0.698 (Fig. 1c), suggesting that even characterising the serotype specificity of the circulating anti-VP1 antibody alone is not a good predictor of disease development.

PsV-specific nAb activity strongly predicts BKV DNAaemia

Since the serotype specificity of the humoral response in the KT pairs was only weakly predictive, we investigated whether the functionality of the humoral response would be a better measure. KT pairs were analysed using PsV neutralisation assays (NA), to define the presence of neutralising antibodies against each serotype. In recipients with significant DNAaemia (n = 28), heatmaps demonstrated lower pre-transplant nAb activity (LogIC50) values compared to the non-viraemic (n = 173) and the low/late DNAaemia cohorts (n = 17; Fig. 2a). Interestingly, we observed that the viraemic cohort also appeared to have lower logIC50 nAb activity than their donors, while the opposite effect was seen in the non-viraemic cohort (Fig. 2b). Having established no difference in demographic data between groups (Table 1) and no significant differences between D/R cohorts' distribution of serotypes neutralised, if seropositive (Chi-Square and Kruskal–Wallis tests or ANOVA where appropriate; Supplementary Fig. S1 and Table S1), we interrogated the responses in more detail. In recipients who developed post-transplant DNAaemia, nAb activity was significantly lower for all the serotypes, with median logIC50 of 2.91 (IQR 1.75–3.48), 1.95 (IQR 1.11–2.51), 1.87 (IQR 0.97–2.33), 1.52 (IQR 1.00–2.30), 1.19 (IQR 0.83–1.60), 1.22 (IQR 0.79–1.65) and 1.20 (IQR 0.83–1.72) for BKV-1a, BKV-Ib2, BKV-Ic, BKV-II, BKV-III, BKV-IVb1 and BKV-Ivc2 respectively. In comparison, median logIC50 for recipients who did not develop BKV DNAaemia were 3.30 (IQR 2.59–3.89), 2.43 (IQR 1.56–2.97), 2.31 (IQR 1.47–2.86), 2.34 (IQR 1.76–2.83), 1.54 (IQR 0.97–2.17), 1.67 (IQR 1.17–2.13) and 1.79 (IQR 1.18–2.21) respectively (one-way ANOVA; Supplementary Fig. S2 and Table S2). In contrast, recipients who developed low level or late BKV DNAaemia, showed no significant difference in median logIC50 values compared to non-viraemic disease controllers (Mann–Whitney U test). These findings suggested that the magnitude of the nAb response was important for control of BKV. Consistent with specific assessment of NA being a better indicator of outcome than total antibody response, there was poor concordance between PsV-specific ELISA and NA data amongst 305 seropositive individuals when subject to several ranking analyses (Supplementary Figs. S3 and S4 and Table S3).

However, we hypothesised that not just the magnitude of the nAb response was important, but also the

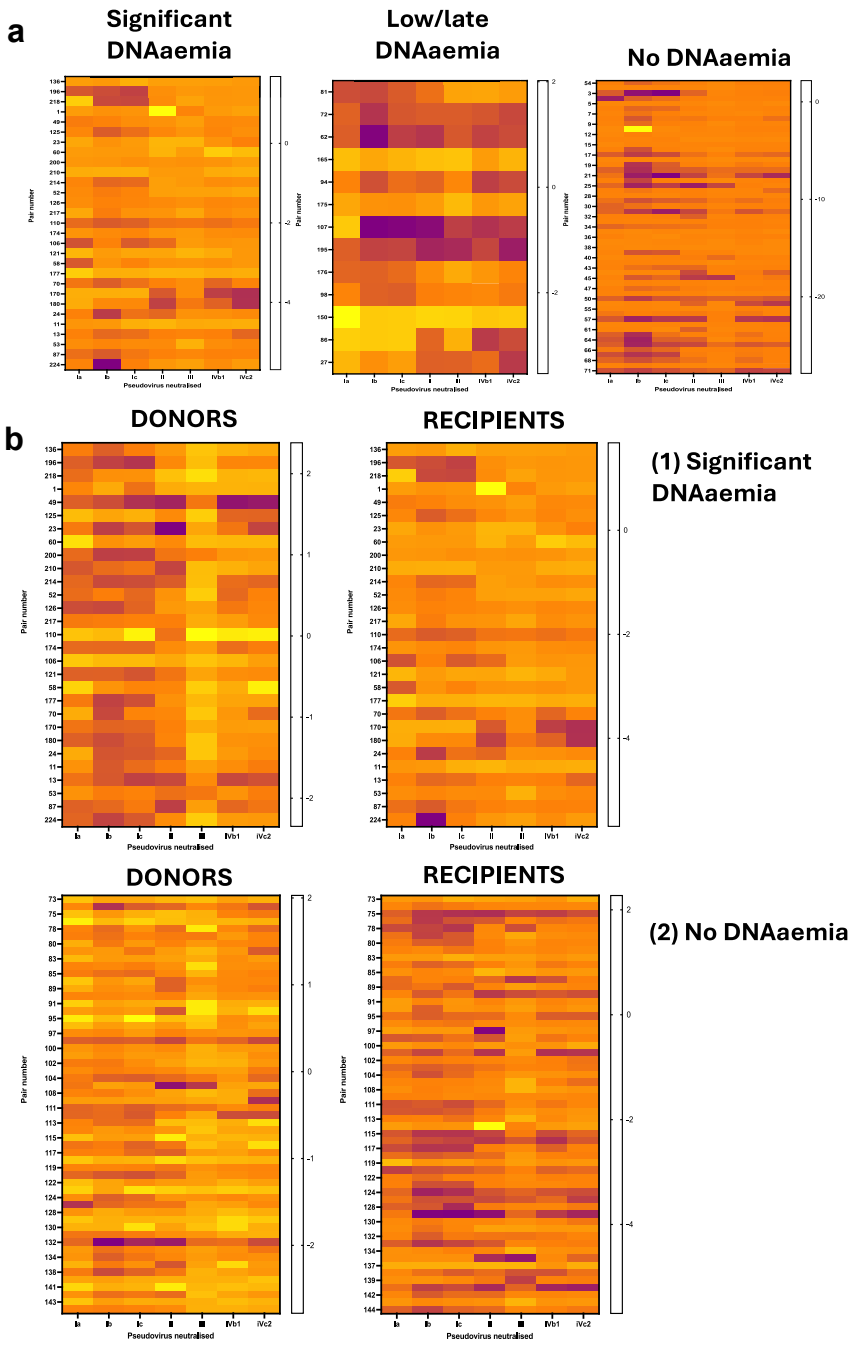

Fig. 2: **(a) Heatmaps showing difference in total neutralisation assay activity for each PsV in recipients from cohorts with significant BKV DNAaemia, low DNAaemia or no DNAaemia**. Heatmaps showing difference in total neutralisation assay activity (LogIC50) relative to the cut-off value for each PsV in individual recipients from cohorts with significant DNAaemia, low DNAaemia or no DNAaemia. KT pairs were categorised into significant DNAaemia of >1000 copies/ml, low level DNAaemia of <1000 copies/ml occurring within 12 months post-transplant and late DNAaemia of >1000 copies/ml occurring after 12 months post-transplant or recipients who did not develop DNAaemia (No DNAaemia). Each row corresponds to an individual recipient's adjusted neutralisation activity against each of the 7 PsVs, corresponding to each column of the heatmap. Recipients with significant post-transplant DNAaemia (left figure) have generally lower relative logIC50 values, compared to recipients with low/late or no DNAaemia (middle and right figures), where lower levels of neutralising activity appear yellow and higher levels of neutralisation appear purple. **(b) Heatmaps of neutralisation assay activity comparing D/R pairs with high DNAaemia vs. no DNAaemia**. Heatmaps showing difference in total neutralisation assay activity (LogIC50) relative to the cut-off value for each PsV in paired donors and recipients from cohorts with **(1)** significant DNAaemia showing lower levels of nAb activity in recipients compared to their paired donors and **(2)** no DNAaemia showing the converse.

specific serotype activity. D/R pairs with post-transplant BKV DNAaemia revealed a greater proportion of donors able to neutralise PsV Ia, Ib2, Ic, II and IVc2, compared to recipients (69.2% vs. 38.9%, 80.8% vs. 61.1%, 73.1% vs. 50.0%, 46.2% vs. 33.3% and 34.6% vs. 22.2% respectively), suggesting a difference in exposure to, and nAb activity against, certain virus genotypes. Although, there was no difference between the groups overall, it suggested that specific nAb activity against different genotypes of BKV, and specifically a mismatch with the infecting strain in the implanting organ, could be important. Detailed analyses of D/R mismatch confirmed that of the 28 KT pairs with significant DNAaemia, 23 (82.0%) had a higher degree of specific NA mismatches, compared to 71 of 190 (37.4%) pairs with late or no BKV disease. ROC curve analysis of NA MM ratios, using cut off >0.67, returned a likelihood ratio of BKV disease of 3.37 ($p \leq 0.001$) with a sensitivity 66.67% (95% CI 47.82–81.36%), specificity 80.65% (CI 74.00–85.23%) and AUC of 0.77 (Fig. 3a).

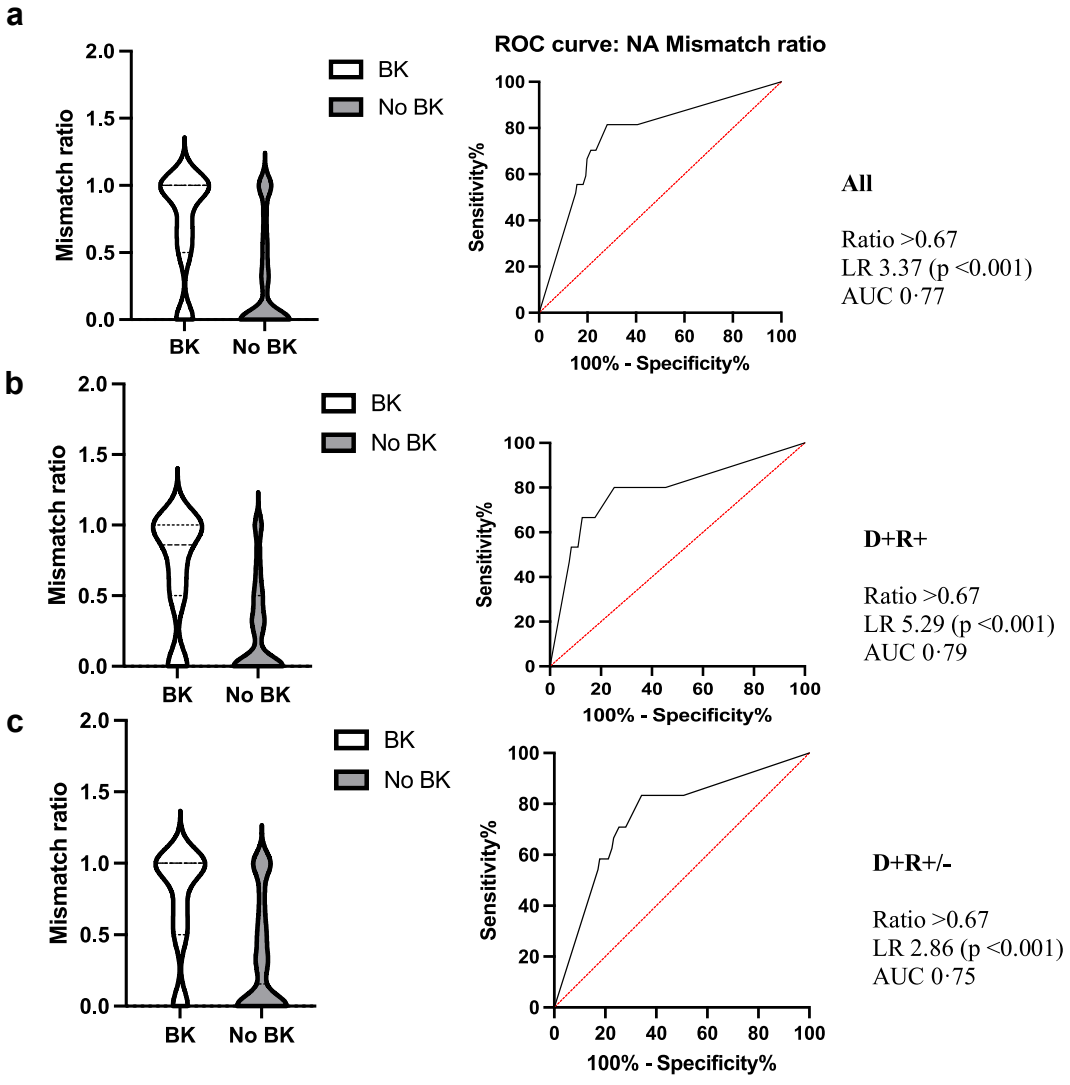

Fig. 3: **Neutralising antibody mismatch ratios (NA MM) and BKV DNAaemia for all kidney transplant pairs and in subsets with sero-positive donors**. Violin plot demonstrating differences in NA MM ratios between viraemic and non-viraemic recipients and ROC curve analysis of NA MM ratios and BKV DNAaemia showing that using a cut-off of 0.67, for all donor and recipient pairs (a) the likelihood ratio of BKV DNAaemia was 3.37 ($p < 0.001$), with a sensitivity of 66.67% (CI 47.82% to 81.36%) and specificity of 80.65% (CI 74.0% to 85.23%) and AUC 0.77. In D+R+ pairs (b), the likelihood ratio of BKV DNAaemia was 5.29 ($p < 0.001$), with a sensitivity of 66.67% (95% CI 41.71–84.82%) and specificity 87.39% (95% CI 80.24–92.21%) and AUC 0.79, and in (c) D + R ± cohort showing that using a cut off NA MM ratio >0.67, the likelihood ratio of BKV DNAaemia was 2.86 ($p < 0.001$), with a sensitivity of 66.67% (95% CI 46.71–82.03%) and specificity of 76.71% (95% CI 69.23–82.83%) and AUC 0.75.

We additionally evaluated only recipients with seropositive donors (D + R ± pairs), where nAb mismatch would be hypothesised to have the most pronounced impact on post-transplant DNAaemia, if infection was donor derived. Individuals were defined seropositive or negative based on the commercial anti-BKV IgG ELISA, or on the NA and PsV-specific ELISA responses. There were 145 D+R+ pairs, of which 17 (11.7%) developed significant BKV DNAaemia. In contrast, 30 pairs were D + R-, and 8 (26.7%) developed significant BKV DNAaemia. There were 9 D-R- pairs, of which 1 (11.1%) recipient developed BKV DNAaemia.

ROC curve analysis was performed on D+R+ pairs. Using cut off >0.67, the likelihood ratio of BKV disease was 5.29 ($p < 0.001$) with a sensitivity 66.67% (95% CI 41.71%–84.82%) and specificity 87.39% (95% CI 80.24–92.21%) and AUC of 0.79 (Fig. 3b). When only the presumed highest risk D + R-pairs were included, there was no further increase in discrimination, but rather, a fall in AUC was noted (Fig. 3c). However, there were 8 D + R-pairs with a NA MM ratio of zero, all of these had a donor who was ELISA seropositive with no evidence of neutralising activity, highlighting the discrepancy between total IgG seropositivity and NA.

Using the ROC curve defined cut offs, a logistic regression analysis was performed to adjust for potential confounding variables. In univariate analysis, NA MM ratio was found to be an independent predictor of BKV disease (odds ratio [OR] 5.84, 95% CI 2.59–13.61) compared to recipients with MM ratios <0.67 (Table 2) and remained associated after adjusting for recipient age, sex, ethnicity and acute rejection (adjusted OR 5.12; 95% CI 2.07 to 13.04). No additional variables were associated with BKV DNAaemia, apart from acute rejection (OR 3.64 [95% CI 1.22 to 10.04]).

Specific NA mismatches are associated with increased risk of BKV DNAaemia

These data argue that D/R mismatches in neutralising activity are important indicators of risk. We next investigated whether the risk of BKV DNAaemia was attributable to specific anti-VP1 D/R mismatches. A mismatch towards PsV Ic or II, where the recipient lacked neutralising activity, was associated with BKV DNAaemia (OR 10.58 [95% CI 4.39–26.08] and OR 4.08 [95% CI 1.71–9.51] respectively), compared to recipients without a Ic or II mismatch (Table 2). Similarly, MM towards PsV Ia, Ib2 and IVb1 were also associated with higher risk of BKV DNAaemia (OR 4.27 [95% CI 1.90 to 9.70], OR 5.15 [95% CI 2.18–12.05] and OR 5.52 [95% CI 2.12–13.97] respectively). After adjusting for recipient age, sex, ethnicity, acute rejection and the other serotypes, MM towards Ic and II remained associated with BKV DNAaemia (adjusted OR 8.12 [95% CI 2.10–35.61] and OR 4.52 [95% CI 1.19–19.23] respectively). However, MM against Ia, Ib2 and IVb1 were no longer associated with BKV DNAaemia (Table 2). The association between serotype mismatch and BKV DNAaemia remained when biopsy proven BKVN (n = 7) was analysed as a secondary outcome, where a MM ratio of >0.67 was associated with BKV DNAaemia (unadjusted OR of 6.86 [95% CI 1.60 to 39.08]; Supplementary Table S4).

Some individuals demonstrate broad neutralisation against all the BKV serotypes

Finally, we observed that among our cohort, 21 recipients and 9 donors had broadly neutralising activity against all the 7 serotypes tested, and none of the recipients developed BKV DNAaemia. Age, sex, ethnicity and transplant characteristics were similar between the recipients who were broadly neutralising and the whole recipient cohort (Supplementary Table S5). Analyses of their humoral responses revealed that regardless of the VP1 serotype that the highest total antibody response was detected against, the majority had their highest nAb titre against BKV-Ia, while almost half the cohort had BKV-III as their highest-ranking total antibody. In contrast, fewer displayed rank 1 nAb responses against other BKV serotypes (Supplementary Fig. S6).

Discussion

In our UK cohort of 224 paired donors and recipients (D/R) of kidney transplants (KT), we found higher rates of BKV DNAaemia in donor seropositive pairs. Overall, D/R mismatch poorly correlates with BKV DNAaemia and cannot realistically be used in clinical practice to risk-stratify patients. We demonstrate that high ratios of D/R mismatch in neutralising activity (nAb) against individual serotypes are superior at predicting post-transplant BKV DNAaemia, compared to simply measuring the total antibody response.

Study limitations include the utilisation of donor neutralising antibody status to infer the likely virus genotypes that may be transmitted. Many individuals demonstrate the ability to neutralise multiple BKV serotypes and without isolating and genotyping, it is difficult to deduce if infection is truly donor derived. Unfortunately, there were insufficient samples from post-transplant viraemic individuals to genotype the replicating virus. However, even if the replicating strain had been identified, Gras et al. showed that only 5 of 6 pre-implantation biopsies had the same BKV strain as that of the replicating strain in the kidney following BKVN diagnosis, although much higher rates of BKVN occurred amongst recipients with BKV detected in donor pre-transplant biopsies.[24] This highlights the challenges of isolating donor BKV, since infection is focal throughout the urinary tract and individuals could be infected with multiple strains of BKV, not detected by

	# with BKV	Univariable			Multivariable[a]		
		OR	95% CI	p value	OR	95% CI	p value
Factors associated with NA MM ratio < 0.67, n = 218							
Recipient factors							
PsV serotype D/R NA MM ratio							
<0.67 (n = 162)		1			1		
>0.67 (n = 56)	16	5.84	2.59–13.61	<0.001	5.12	2.07–13.04	<0.001
Age (years)							
<60 (n = 152)		1			1		
≥60 (n = 66)	8	0.94	0.38–2.15	0.89	0.98	0.34–2.50	0.96
Sex (male vs. female)	21	2.13	0.92–5.47	0.08	1.88	0.73–5.42	0.20
Recipient ethnicity							
White (n = 79)		1			1		
Non-white (n = 112)	19	1.45	0.64–3.50	0.38	1.37	0.55–3.58	0.50
Transplant type							
Live (n = 21)		1					
DBD (n = 138)	18	0.90	0.24–3.37	0.88			
DCD (n = 59)	7	0.81	0.19–3.46	0.77			
BMI							
≤25 (n = 87)		1					
>25 (n = 130)	14	0.63	0.28–1.40	0.25			
HLA mismatches							
≤3 (n = 155)		1					
>3 (n = 63)	7	0.80	0.32–1.98	0.63			
Pre-emptive transplant (n = 40)	5	0.82	0.29–2.28	0.68			
Corticosteroid use (n = 95)	15	1.57	0.71–3.49	0.27			
CMV DNAaemia >2000 copies/ml (n = 71)	11	1.39	0.61–3.15	0.43			
Acute rejection <12 months (n = 20)	6	3.64	1.22–10.04	0.02			
Donor factors							
Age (≥ 60; n = 69)	11	0.98	0.96–1.01	0.26			
Sex (male; n = 132)	20	1.65	0.69–3.97	0.26			
Donor ethnicity							
White (n = 194)		1					
Non-white (n = 23)	5	1.57	0.49–5.01	0.47			
Mismatched PsV					**Adjusted to other PsVs[a]**		
Ia (n = 55)	15	4.27	1.90–9.70	<0.001	1.52	0.42–4.92	0.50
Ib2 (n = 36)	12	5.15	2.18–12.05	<0.001	1.07	0.22–4.30	0.93
Ic (n = 30)	14	10.58	4.39–26.08	<0.001	8.12	2.10–35.61	0.002
II (n = 37)	11	4.08	1.71–9.51	0.002	4.52	1.19–19.23	0.03
III (n = 22)	3	1.21	0.30–3.67	0.76	0.17	0.02–0.93	0.04
IVb1 (n = 24)	9	5.52	2.12–13.97	<0.001	2.54	0.52–13.93	0.25
IVc2 (n = 45)	9	2.06	0.85–4.76	0.11	1.33	0.24–5.59	0.72

Likelihood ratio tests were used to assess the strength of association at each level. [a]Adjusted for age, sex, ethnicity and acute rejection.

***Table 2*: Firth logistic regression models for the incidence of BKV DNAaemia (>1000 copies/mL within the first-year post-transplant) for NA MM ratio greater than 0.67 vs. less than 0.67 and for mismatches against specific donor PsV serotypes compared to those with no mismatch against the corresponding serotype.**

a single biopsy. Without genotyping donor BKV, it is difficult to ascertain if the post-transplant infection is truly donor derived. As BKV genomes are often difficult to isolate from tissue or blood in immunocompetent individuals, these data still need be incorporated with nAb data in order to be meaningful. Furthermore, although the use of neutralisation assays has been used extensively to study D/R BKV serotypes, it is difficult to differentiate cross-neutralisation and co-infection with multiple strains. The latter is likely to be a common due to high rates of migration. Indeed, a Spanish study found that half of all viraemic KT recipients were found to have mixed genotypes.[27] It has also been shown that cross-neutralisation is seen between genotypes,

particularly BKV-Ib1 (and Ia which differs from Ib1 in a region outside of the VP1 genome) and BKV-Ib2, which appears to have significant cross-reactivity with BKV-Ic, and between the BKV-II and BKV-III genotypes and BKV-IV genotypes.[4] However, by using the totality of the nAb mismatch, our analysis takes this into account and instead, negates the need to know the genotype from a clinical perspective. A further limitation was the small numbers of individuals with BKV DNAaemia $>10^4$ copies/ml (n = 14) and with BKVN (n = 7) in our cohort. Our unit uses an IL-2 antagonist induction strategy with early steroid withdrawal in over half the cohort, paired with a robust monitoring and intervention strategy, and this likely explains the lower prevalence of high level DNAaemia. Therefore, a caveat of these studies is that we define clinically significant DNAaemia as $>10^3$ copies/ml, whereas the American society of transplantation (AST) defines presumed BKVN as $>10^4$ copies/ml.[28] This may over-estimate the impact on BKVN. However, several studies demonstrate this is underestimating BKVN diagnoses, with ~35% of biopsy-proven BKVN cases having corresponding viral loads of $<10^4$ copies/ml and with outcomes similar to those with higher viral loads.[29,30] Regardless, we are unable to determine if MM ratios are predictive of BKVN in this cohort.

A key outcome of this study demonstrates that the nature of the humoral response against BKV in the recipient is an important factor, particularly in the context of how it mismatches with the organ donor. Indeed, the differential capacity of individual recipients to control an incoming strain of BKV potentially explains the high rate of discordance in BKV infection seen between kidneys transplanted from one donor into two separate individuals.[31] Bae et al. recently demonstrated the use of BKV serostatus, combined with BKV T-cell reactivity to predict risk,[32] however, serotyping alone has not been shown to be predictive of disease. We show that a nAb assessment is superior to an ELISA based approach. In agreement with Solis et al.,[23] we observe that intensity of the nAb response is associated with disease control, however amongst non-viraemic disease controllers, we observed median logIC50 values of 2.13–3.30, much lower than the $>4\log_{10}$ cut-off quoted in their paper. Additionally, we demonstrate that greater degrees of mismatched neutralising antibodies between donors and recipients were associated with higher likelihood of DNAaemia, but also identify that not all MM are associated with equivalent risk. Specifically, the highest risk is seen in individuals with mismatches against donor Ic or II serotypes. This could suggest that these are more virulent genotypes and indeed, Gras et al. found 25% of genotyped viruses from BKVN biopsies were BKV-II genotype, however genotype Ic was only found in 2 of 32 samples.[24] Therefore, the more likely conclusion is that this reflects the difference in genotypes present in our recipient and donor populations and the lack of recipient specific humoral immunity, relative to the donor. In our cohort, we have found much higher rates of neutralising activity against different serotypes, with average donor and recipients nAb prevalence of 39%, 58%, 50%, 40%, 11%, 23% and 30% towards BKV-Ia, Ib2, Ic, II, III, IVb1 and IVc2 respectively (Supplementary Fig. S1). This is significantly higher than that in previous studies,[23] which could explain why we have observed higher risk with BKV-Ic and BKV-II mismatches and could be explained by the high ethnic diversity amongst recipients (Supplementary Table S1) where 63.8% were "Non-White" (n = 143) and conversely, in the donor group, 89.3% were "White" (n = 200). Although findings were not significant, we observed differences in D/R nAb responses; a larger proportion of sera from "White" individuals showed no neutralising activity against any of the serotypes, while greater neutralising activity against serotype III was seen in the "Non-White" cohort (Chi-square; Supplementary Fig. S5). This suggests ethnicity may be an important contributing factor to D/R serotype mismatch. Recently, a large cohort study in Australia and New Zealand found that D/R ethnicity mismatch was associated with increased risk of BKVN,[33] while another UK study observed a nearly 3-fold increase in risk of BKV DNAaemia in Afro-Caribbeans.[34] These findings were not supported by our study, but this was likely due to lack of power, incomplete recording of ethnicity, and lack of detail on the country of birth when initial BKV exposure may have occurred.

In conclusion, we have described the relationship between D/R mismatches and post-transplant BKV DNAaemia, using different means of defining antiviral humoral immunity. We have demonstrated the utility of pre-transplant BKV nAb assessment, which has the potential to be developed for regional or national level testing peri-transplantation and allow for post-transplant risk prediction and individualised treatment modification. Furthermore, we identified a strong risk phenotype when a serotype Ic or II mismatch occurred suggesting that specific serotypes of BKV may represent greater risk in our local populations. We have also identified individuals with broadly neutralising sera who appear to control disease which we believe warrants further investigation, and may lead to promising therapeutic options based on several recent studies in this area.[35–37]

Contributors

The project was conceived and designed in collaboration with Dr Mark Harber and Dr Ciara Magee in the kidney transplant unit at the Royal Free hospital and with Stephanie Chong's PhD supervisors Dr Matthew Reeves & Prof Alan Salama. Both supervisors were also instrumental in funding acquisition. Identification and recruitment of patients and

collection and processing of samples was done by the first author, with the help of Dr Yuen Chang. Stephanie Chong also applied for, and instigated, the use of QuOD samples to increase the cohort size. Dr Raymond Fernando identified and collected stored recipient samples from the Anthony Nolan laboratories. All the experiments were carried out independently, including the production and purification of BKV pseudoviruses and the design and optimisation of the pseudovirus-specific ELISA and neutralisation assays, with the help of Dr Claire Atkinson in the optimisation of ELISA assays. Acquisition and collation of all relevant clinical data and analysis of the results was performed by Stephanie Chong with the guidance of the supervisors listed above. Dr Rachel Hung contributed by producing the R studio coding for the logistic regression. The literature search, production of figures and tables and writing of the manuscript was done by Stephanie Chong. Dr Reeves and Dr Chong have accessed and verified the data and Dr Hung has also accessed and verified the data in the logistic regression models. Dr Reeves and Prof Salama both also contributed to review and editing of the manuscript. All authors read and approved the final version of the manuscript.

Data sharing statement

Data collected and analysed for this study is available upon request from the corresponding authors: Dr Stephanie Chong: Stephanie.chong1@nhs.net. Prof Alan Salama: a.salama@ucl.ac.uk. Dr Matthew Reeves: matthew.reeves@ucl.ac.uk.

Declaration of interests

Stephanie Chong was granted funds by HCA healthcare UK to present this work at the British Transplantation Society Annual Congress 2024. The other authors make no further declarations of interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2024.105430.

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