Bachelor of Science in Medicine Degree Program
End of Term Final Report

Office of Graduate and Advanced Degrees
Education in Medicine Max Rady College of Medicine
Rady Faculty of Health Sciences
University of Manitoba

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Project Title: Safety and efficacy of a reduced frequency post-transplant monitoring strategy for EBV, CMV, and BKV.

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Summary (250 words max single spaced):
BACKGROUND: There is a lack of consensus on optimal viral monitoring protocol for kidney transplant recipients. In response to the increased demand on laboratory testing during the COVID-19 pandemic, the Transplant Manitoba Adult Kidney Program (TMAKP) adopted reduced frequency monitoring protocols for cytomegalovirus (CMV), Epstein Barr virus (EBV), and BK polyomavirus (BKV).
METHODS: This single-center retrospective observational cohort study evaluated 341 adult kidney transplant recipients transplanted between January 2015 to March 2021, with the change in protocol effective on March 19th 2020. Recipients transplanted prior to March 19, 2019 comprised the pre-protocol cohort, while those transplanted after March 19, 2020 were contained in the post-protocol cohort. For each viremia, an incident cohort consisting of recipients transplanted post-protocol change who developed viremia were matched to historical controls from the pre-protocol era. The prevalent cohort consisted of recipients transplanted in the pre-protocol period with established viremia prior to the protocol change.
RESULTS: There were no significant differences in maximum viral load between incident cases and controls in titers of EBV, CMV, or BKV. Among recipients with established viremia pre-protocol change, and who had at least one viral load checked post-protocol change, median viral load was significantly lower post-protocol in the CMV (860 vs 0 copies/mL, $p<0.0001$) and BKV (26500 vs 500 copies/mL, $p<0.0001$) prevalent cohorts, but not the EBV cohort.
CONCLUSION: Our findings suggest that reduced frequency monitoring may be safe and cost-effective.

S[redacted]ure Primary Supervisor Signature

Ackno[redacted]nts: I gratefully acknowledge the sole or partial funding support from:

H.T. Thorlakson Foundation
Dean, College of Medicine
Research Manitoba

Manitoba Medical Service Foundation (MMSF)
Vice-Dean, Research Rady FHS
Health Sciences Centre Research Foundation
Heart and Stroke Foundation

Introduction

Kidney transplant recipients must balance immunosuppression needed to prevent rejection with risks of over-immunosuppression including infection. Cytomegalovirus (CMV), Epstein Barr virus (EBV), and BK polyomavirus (BKV) have high seroprevalence in the general population where these infections are typically asymptomatic or subclinical. In contrast, severe disease occurs more commonly in kidney transplant recipients, with CMV disease in 5%, post-transplant lymphoproliferative disease (PTLD) in 0.3-2%, and BKV nephropathy (BKVN) in 5-10%, where they confer worse graft and patient survival(1-3). With the exception of CMV, there is a lack of targeted antiviral therapy, and treatment is typically limited to non-standardized reduction of overall immunosuppression. However, this approach is not without consequence as immunosuppression reduction for treatment of viremia has been associated with increased risk of acute rejection.

In the absence of effective or evidence-based treatments for established disease, existing guidelines highlight the need for early detection and treatment of viremia where indicated. However, a lack of randomized controlled trial data has resulted in no clear international consensus on optimal surveillance strategies, and available recommendations are often limited to expert opinion (4-6). There is also a paucity of published real-world data from the experiences of transplant programs, among which there is significant practice variation with respect to frequency, duration, and even modality of testing (7). This places a significant responsibility on transplant programs to audit and validate their surveillance strategies against important clinical outcomes in real-world settings. This is especially important when programs implement changes to their surveillance strategies, so as to ensure clinical performance is preserved while not causing undue burden to patients or financial cost to the healthcare system. There is a need for increased transparency and reporting of institutional protocols and their outcomes.

The SARS-CoV-2 (COVID-19) pandemic imposed unprecedented strains on the healthcare system requiring new crisis standards of care and rationing of critical resources to expand and prioritize COVID-19 testing capacity (8,9). To minimize its impact on the Cadham Provincial Lab, the Transplant Manitoba Adult Kidney Program (TMAKP) revised its post-transplant viral monitoring protocols to decrease the frequency of viral testing while maintaining alignment with 2018-2019 international recommendations on post-kidney transplant surveillance for CMV, EBV, and BKV. (4-6). With this retrospective cohort study, we sought to evaluate the safety and effectiveness of the reduced frequency viral monitoring protocol in adult kidney transplant recipients by examining the maximum viral loads of EBV, CMV, and BKV before and after changes to the frequency of post-transplant viral monitoring.

Methods

Study Design and population

The study received ethics approval from the University of Manitoba Health Research Ethics Board (HS24719, H2021:095). This single-center retrospective cohort study consisted of 341 consecutive adult kidney transplant recipients transplanted from January 2015 to March 2021 from Transplant Manitoba. Recipients were excluded for primary non-function, death within 30 days of transplant, or transplant occurring between March 19, 2019 and March 18, 2020 due to overlap in viral monitoring protocols during the first year post-transplant. Standard triple maintenance immunosuppression was tacrolimus, mycophenolic acid, and prednisone.

Demographic data, medication use, and clinical outcomes were extracted from the TMAKP electronic medical record (EMR). Viral load data were extracted from the TMAKP EMR and the Cadham Provincial Laboratory database.

Viremia monitoring protocols

Transplant Manitoba protocols for viral screening, diagnosis, treatment, and post-treatment monitoring are detailed in the Supplemental Methods. Briefly, recipients are risk-stratified for developing CMV infection based on donor-recipient serologic mismatch and thymoglobulin induction with positive recipient serology. In the standard protocol, recipients at high and moderate risk of CMV underwent weekly testing for the first 6 months post-transplant, as well as for 3 months following treatment of rejection with thymoglobulin. Screening was extended beyond 6 months post-transplant in high-risk recipients, with testing every 2-4 weeks until 1-year post-transplant. The revised protocol foregoes testing in high-risk recipients while receiving universal prophylaxis with valganciclovir for the first 6 months post-transplant or after treatment of rejection with thymoglobulin. Biweekly screening is resumed for 3 months after discontinuing prophylaxis. High-risk recipients who receive only steroids for treatment of rejection are screened for 3 months. Moderate risk recipients are screened for the first 6 months post-transplant with biweekly testing if they received basiliximab induction or monthly testing if they received no induction. The revised protocol also specifies that CMV PCR should be monitored biweekly while receiving valganciclovir treatment, and for 2 months after discontinuation of valganciclovir. In both protocols, CMV PCR should be checked if clinically indicated.

Recipients are risk-stratified for developing EBV viremia based on donor-recipient serologic mismatch. In the standard protocol, high-risk recipients were screened monthly for the first year post-transplant, once after treatment of rejection, and if clinically indicated. The revised protocol reduces monitoring frequency to every 3 months starting from 6 months post-transplant and if clinically indicated.

BKV monitoring in the standard protocol included weekly urine decoy testing for the first 6 months and every 2-4 weeks until 1-year post-transplant. In the revised protocol, testing is done biweekly for the first 3 months post-transplant, monthly till 6 months post-transplant, and then every 3 months till 2 years post-transplant. In both protocols, BKV PCR is done following positive urine decoy result, or if clinically indicated.

Study cohorts

The minimized viral monitoring protocol was implemented on March 19, 2020. Recipients transplanted prior to March 19, 2019 comprised the pre-protocol cohort, while those transplanted after March 19, 2020 were contained in the post-protocol cohort. For each viremia, an incident and prevalent cohort were identified. The incident cohort consisted of all recipients transplanted after March 19, 2020 who developed viremia. The prevalent cohort included patients transplanted before March 19, 2019 who had an established history of viremia prior to the protocol change on March 19, 2020.

Outcomes of interest

The study's primary outcome was the difference in maximal viral load magnitude between post-protocol incident cases and matched pre-protocol historical controls as a surrogate for tissue invasive disease. Where available, secondary outcomes in the incident cohort included first viral load magnitude, time to first viral load, clearance of viremia determined by achieving negative PCR, sustained clearance of viremia with at least two negative PCR results at least one week apart, and time to clearance of viremia after initial viremia detection. Another secondary outcome in the prevalent cohort was the difference in viral load magnitude between the post-protocol and pre-protocol time periods. Safety outcomes of CMV disease, PTLD, and BKV nephropathy were also determined where available.

Statistical Analysis

Analyses were conducted using JMP Pro (Version 15.2.0. SAS Institute Inc., Cary, NC). Descriptive statistics were done with categorical variables presented as frequency and percentage and tested using Chi-squared and Fisher exact tests. Continuous variables were presented as median and interquartile range (IQR) and tested using Wilcoxon or Kruskal-Wallis rank sum tests. A two-tailed p-value < 0.05 was considered significant.

Incident cohort cases were matched to pre-protocol controls who developed viremia. Matching criteria was based on recipient age at transplant alone for BKV, and also high risk for viremia based on thymoglobulin induction and donor-recipient serologic mismatch defined as donor positive IgG and recipient negative IgG for CMV and EBV. Cases were matched to controls in a 1:2 fashion for CMV, 1:3 for BKV, and 1:6 for EBV.

Maximal viral loads of prevalent recipients in the post-protocol era was compared to their historic maximal viral loads in the pre-protocol era.

Results

The overall study population consisted of 270 adult kidney transplant recipients eligible for analysis. The post-protocol cohort consisted of 40 recipients transplanted between March 19, 2020 and March 20, 2021 and the baseline demographics were similar to the pre-protocol cohort of 230 recipients transplanted between January 01, 2015 and March 18, 2019. (Table 1). Median follow-up was significantly longer in the pre-protocol cohort (3.8 vs 0.73 years, $p<0.0001$).

Epstein-Barr virus incident cohort

EBV viremia was detected in 52/230 (23%) recipients in the pre-protocol cohort and 6/40 (15%) recipients in the post-protocol cohort, with the latter comprising the "EBV incident cohort". (Figure 1a) Recipient demographics for the EBV incident cohort cases and the 36 matched historical controls from the pre-protocol cohort are shown in Table 2a. There were no significant differences between the EBV incident cohort cases and matched controls in titers of maximum (1340 vs 3650 copies/mL, $p=0.80$) or first (814 vs 288 copies/mL, $p=0.37$) viral loads. Compared

to matched controls, incident post-protocol cases had significantly shorter time to first positive viral load (19 vs 181 days, p = 0.0013). There were no significant differences in proportion of recipients who achieved a negative PCR, two negative PCRs at least 1 week apart, nor in time to viremia clearance. (Table 2b).

Epstein-Barr virus prevalent cohort

There were 45 recipients who developed EBV viremia before March 19, 2020. Three patients were excluded due to graft failure before March 19, 2020, leaving 42 patients in the "EBV prevalent cohort". (Figure 1b) There were 25/42 (60%) recipients who had EBV PCR checked post-protocol change and EBV viremia was detected in 23/42 (55%) recipients. Among recipients with EBV viremia detected pre-protocol change and who had at least one viral load checked post-protocol change, there was no significant difference in median viral load post-protocol change (6130 vs 1125 copies/mL, p=0.1297). Analysis of PTLD was not possible as there were no new PTLD cases identified post-protocol change.

Cytomegalovirus incident cohort

There were 175 recipients who developed CMV viremia during the study period, including 24/40 (60%) post-protocol incident cases in the "CMV incident cohort" and 151/230 (66%) recipients pre-protocol change. (Figure 2a) Recipient demographics for the CMV incident cohort cases and the 48 matched controls from the pre-protocol cohort are shown in Table 3a. There were no significant differences between the CMV incident post-protocol cases and matched controls in titers of maximum (342 vs 893 copies/mL, p = 0.41) or first (138 vs 138 copies/mL, p=0.10) viral loads. There was no significant difference in time to first viral load. Although there was a trend to longer time to negative PCR in the CMV post-protocol incident cases (46 vs 23 days, p=0.055), there was no difference in the proportion of recipients who obtained a negative PCR, two consecutive negative PCRs at least one week apart, or the time to two consecutive negative PCRs at least one week apart. (Table 3b)

Cytomegalovirus prevalent cohort

The "CMV prevalent cohort" consisted of 150 recipients who developed CMV viremia before March 19, 2020. (Figure 2b) There were 59/150 (39%) recipients who had CMV PCR checked post-protocol change and CMV viremia was detected in 23/150 (15%) recipients. Among recipients with CMV viremia detected before the protocol change and who had at least one viral load checked post-protocol change, the median viral load was higher in the pre-protocol era (860 vs 0 copies/mL, p<0.0001). There were 6 prevalent patients who developed tissue invasive CMV disease pre-protocol change and 1 prevalent patient who developed CMV disease post-protocol change.

BK virus incident cohort

There were 98 recipients who developed BKV viremia during the study period, including 12/40 (30%) post-protocol incident cases in the “BKV incident cohort” and 86/230 (37%) recipients pre-protocol change. (Figure 4a) Recipient demographics for the BKV incident cohort cases and the 36 matched controls are shown in Table 4a. There were no significant differences between the BKV incident post-protocol cases and matched controls in titres of maximum (78500 vs 18000 copies/mL, p = 0.44) or first (1650 vs 1200 copies/mL, p=0.65) viral loads. There was no significant difference in time to first viral load, time to negative PCR, or in the proportion of recipients achieving a negative PCR. Although there was a trend to fewer recipients obtaining two negative PCRs at least 1 week apart in the BKV post-protocol incident cohort cases (50% vs 81%, p=0.061), this was not significant and there was no difference in time to sustained viremia clearance.

BKVN was identified in 8 (67%) of the post-protocol incident cohort cases and 30 (83%) of the pre-protocol matched controls (p=0.24).

BK virus prevalent cohort

There were 79 recipients who developed BKV viremia before March 19, 2020. Five patients were excluded due to graft failure before March 19, 2020, leaving 74 patients in the “BKV prevalent cohort”. (Figure 4b) There were 65/74 (88%) recipients who had BKV PCR checked post-protocol change and BKV viremia was detected in 41/74 (55%) recipients. Among recipients with BKV viremia detected before the protocol change and who had at least one viral load checked post-protocol change, the median viral load was higher in the pre-protocol era (26500 vs 500, p<0.0001). There were 17 recipients in the BKV prevalent cohort who developed BKVN pre-protocol change, and there were no cases detected post-protocol change.

Discussion

In this retrospective analysis comparing standard and reduced frequency viremia monitoring protocols for kidney transplant recipients, there was no difference in maximum or first viral load titers among recipients with EBV, CMV, or BKV viremia detected. EBV viremia was detected earlier in recipients surveilled with the reduced frequency protocol. Notably, there was no difference in proportion of recipients achieving viremia clearance nor in time to viremia clearance. Among recipients with established viremia pre-protocol change, and who had at least one viral load checked post-protocol change, median viral load was significantly lower post-protocol in the CMV and BKV prevalent cohorts. Our results suggest the use of a reduced frequency viral monitoring protocol may be a viable, effective, and safe strategy.

Viral load magnitude has variably been associated with increased risk of viral disease. This association is perhaps best established among recipients with high BKV viral load, where titers exceeding 10,000 copies/mL are considered presumed BKV nephropathy. In contrast, the presence of EBV viremia has poor positive predictive value for PTLD, and may not be present in up to 30% of PTLD cases in some series. Nonetheless, in the absence of more valid biomarkers of viral disease, monitoring for viremia remains integral to the management of post-transplant viral disease which lack effective targeted treatments. Although our study was under-powered to detect differences between tissue-invasive viral disease, due to the short follow-up of the post-

protocol period and overall low incidence of these events, it is reassuring that recipients' maximal viral load magnitude was not significantly different between the post-protocol incident cases and pre-protocol matched controls.

There was also no difference in first viral load magnitude for any of the incident viremia cohorts. This suggests that viremia detection was not delayed in recipients undergoing the reduced frequency protocol. In fact, the time to first viral load was significantly shorter among EBV incident cases compared to matched controls. It is possible that this was due to lower adherence to the previous monitoring protocols. Following viremia detection, there was also no difference in measures of viremia clearance which is ultimately a goal of a viremia monitoring protocol. While we were unable to comment on viremia recurrence, we attempted to account for known fluctuations in viremia levels by assessing sustained viremia clearance with at least two negative viral PCRs at least one week apart. This was a reasonable benchmark as it is generally considered a criteria for discontinuation of treatment for viremia. We also observed lower median viral load in the post-protocol period for the CMV and BKV prevalent cohorts, suggesting the effective treatment of viremia in these recipients. The median viral load in the EBV prevalent cohort remained at a low titer in the post-protocol period. This study was intended to provide early quality assurance and identify signals of loss of efficacy or increased adverse outcomes in recipients undergoing the reduced frequency protocol. This monitoring should be extended as an ongoing initiative in the TMAKP to detect any adverse long-term clinical outcomes associated with this change in protocol. The lack of RCTs to inform consensus guidelines on optimal surveillance protocols for viremia following kidney transplant creates a need for programs to undertake quality assurance initiatives such as this, especially when implementing protocol changes. Moreover, there is also a lack of established quality metrics which programs should seek to achieve with respect to viremia. Future studies describing the experiences of individual transplant programs may facilitate creation of such definitions for standard of care.

The reduced frequency protocol brings the TMAKP into greater alignment with existing guidelines. The 2019 American Society of Transplantation Guidelines on EBV suggest screening high-risk recipients, defined by donor-recipient EBV serologic mismatch, at regular intervals in the first year post-transplant. However, in a recent survey of European transplant centers, 38% of centers perform routine monitoring of all transplant recipients, regardless of risk factors for PTLD. (10) This highlights the difference in real-world practice and guidelines, as well as a general tendency for over-utilization of viral testing with variation among transplant centers. Screening for BKV viremia similarly lacks consensus among transplant centers where a survey of American clinicians reported the use of blood PCR alone in 42%, urine alone in 17%, and both in 37%.(11) The British Columbia Transplant program screens for BKV PCR in blood biweekly for 4 months and then monthly for 2 years. Our reduced frequency protocol follows current ATS guidelines which suggest that most cases of BKVN can be captured by screening urine cytology up to 2 years post-transplant, and if detected, followed by testing for BKV PCR(6). For detection of CMV infection and reduction of CMV related complications, there are comparable safety outcomes with a preemptive viral monitoring approach versus universal prophylaxis with valganciclovir. There is no evidence to support routine viral monitoring for CMV while receiving prophylaxis and this change was implemented in the reduced frequency protocol(4). Future studies may explore outcomes of viral monitoring post-prophylaxis, as well as the incidence of late-onset CMV disease.

Laboratory resources and healthcare are a limited resource, and cost is an important consideration. Using the cost per test for each viremia from Cadham Provincial Laboratory and

comparing the reduced monitoring protocol from the prior protocol, we can estimate the savings on resources. For patients at high risk for CMV infection, there would be 26 and 33 less tests needed by the 6 months and the 12 months period, respectively, totaling cost-savings of $992.16 and $1259.28. As for moderate risk patients who have undergone basiliximab induction, there would be 7 and 17 less tests needed by 6 and 12 months, totaling cost-savings of $267.12 and $648.72. For moderate risk patients with no induction, there would be 20 less tests needed by 6 months with cost-savings of $763.2. For EBV monitoring, there would be no difference in testing in the first 6 months however there would be 4 fewer tests performed by 12 months leading to a savings of $208.64. In monitoring for BKV, the reduced frequency would lead to 17 and 28 less tests by 6 and 12 months, with cost-savings of $1706.8 and $2811.2. The reduced frequency protocol may yield significant cost-savings in the long-term. There is also potential unmeasured benefit to patients from reduced bloodwork with respect to decreased time away from work and home, decreased cost of travel to phlebotomy labs, reduced blood loss from blood draws, and decreased psychosocial burden of medical procedures. There was also potential benefit from reduced exposure to healthcare settings during the COVID-19 pandemic, at a time when nosocomial transmission of COVID-19 was high.

This study has several limitations. There are inherent limitations to the study design being a single-center, retrospective study. The evaluated post-protocol period was short, thereby limiting the post-protocol cohort size and it is possible that later cases of viremia were not captured here. However, the first year post-transplant is considered the highest risk period for viremia occurrence given that this is when recipients generally receive the greatest degree of immunosuppression. The study was underpowered to assess CMV disease, PTLD, or BKVN, given the small sample size, short follow-up period, and overall low incidence of these diseases. Finally, although there were standard viral monitoring protocols in place, the adherence to the testing schedule was not assessed.

The strengths of the study are as an exploratory quality assurance project that addresses an important area for future study. There is a lack of literature validating viral monitoring frequencies with clinical guidelines that are often based on expert opinion and varying clinical practices between transplant centers (5,6). The TMAKP provides a closely tracked and well-annotated cohort of adult kidney transplant recipients. The Cadham Provincial Laboratory also provides a complete and robust database of viral testing performed. This study provides real-world benefits in validating reduced viral monitoring which includes a reduction in cost and patient burden.

The updated viral monitoring approach is safe and effective and overall cost-savings could be gained from the reduced frequency protocol in the long-term. Additional exploratory studies can be undertaken in the pediatric kidney transplant, and lung and liver transplant populations in Transplant Manitoba as they made similar accommodations to their post-transplant viral monitoring protocols during the pandemic. This project should be extended on an ongoing basis by the TMAKP to detect longer-term outcomes.

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Tables and Figures

Table 1. Baseline demographics of pre-protocol (n=230) and post-protocol (n=40) cohorts

Baseline and perioperative variables	**Pre-protocol cohort (n=230)**	**Post-protocol cohort (n=40)**	**P-value**
Age at transplant (yrs) (n=270)	52.5 (37.8, 62.3)	50.3 (41.6, 61.4)	0.9764
First transplant (%)	206 (90%)	35 (88%)	0.7812
Male (%) (n=269)	146 (64%)	30 (77%)	0.3945
Caucasian (%) (n=270)	140 (61%)	18 (45%)	0.0812
Living donor (%) (n=270)	103 (45%)	13 (33%)	0.2218
PRA	2.5 (0, 59.3)	0 (0, 18.5)	0.0830
Alloimmune risk category (%) (n=270) - Low - Intermediate - High	 48 (21%) 89 (39%) 93 (40%)	 14 (35%) 8 (20%) 18 (45%)	**0.0397**
Pre-transplant DSA (%) (n=267)	15 (6%)	1 (3%)	0.4800
Induction (%)	176 (77%)	30 (75%)	0.8415
Induction agent - None - Basiliximab - Thymoglobulin	 54 (23%) 97 (42%) 79 (34%)	 10 (25%) 27 (68%) 3 (8%)	**0.0016**

Figure 1a. Patient flow for the EBV incident cohort

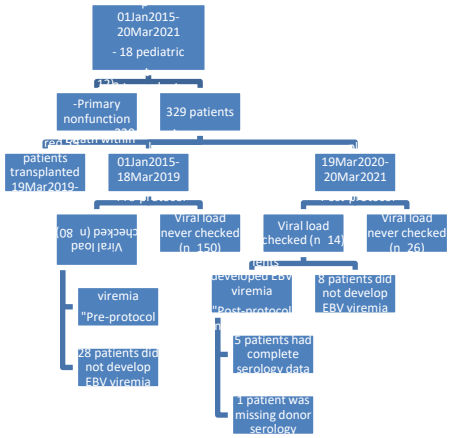

Table 2a. Baseline demographics of EBV viremic patients transplanted after 19Mar2020 (post-protocol incident cohort cases) (n=6) compared to patients transplanted 01Jan2015-19Mar2019 (matched pre-protocol controls) (n=36)

Baseline and perioperative variables	Controls n=36	Cases n=6	P-value
Age at transplant (yrs) (n=42)	41.1 (31.3, 64.4)	44.6 (22.3, 54.6)	0.8433
First transplant (%) (n=42)	32 (89%)	5 (83%)	0.5568
Alloimmune risk category (%) (n=42) - Low - Intermediate - High		missing	
Pre-transplant DSA (%) (n=42)	4 (11%)	0 (0%)	1.0000
PRA (n=42)	0	0.5	0.8308
Induction (%) (n=42)	32 (89%)	4 (67%)	0.1975
Thymoglobulin (%) (n=42)	10 (28%)	1 (17%)	1.0000
EBV donor/recipient serology (n=41) - Neg/neg - Neg/pos - Pos/pos - Pos/neg	36 (100%) 0 1 (3%) 20 (56%) 15 (42%)	5 (83%) 0 0 2 (33%) 3 (50%)	0.6856

Table 2b. Outcomes of EBV viremic patients transplanted after 19Mar2020 (post-protocol incident cohort cases) (n=6) compared to patients transplanted 01Jan2015-19Mar2019 (matched pre-protocol controls) (n=36)

Outcome	Controls (n=36)	Cases (n=6)	P-value
Time to first viral load (dys) (n=42)	181 (91, 682)	19 (8, 54)	**0.0013**
First viral load (copies/mL) (n=42)	288 (288, 1055)	814 (288, 5335)	0.3684
Max viral load (copies/mL) (n=42)	3650 (288, 52000)	1340 (288, 47400)	0.8028
Negative PCR achieved (%) (n=42)	19 (53%)	2 (33%)	0.6628
Time to first negative PCR (dys) (n=21)	42 (13.4, 97)	49 (14, 84)	1.0000

Two consecutive negative PCRs at least 1wk apart achieved (%) (n=42)	10 (28%)	1 (17%)	1.0000
Time to Two consecutive negative PCRs at least 1wk apart (dys) (n=11)	33.7 (20.0, 123.8)	23 (23, 23)	0.8744

Figure 1b. Patient flow for the EBV prevalent cohort

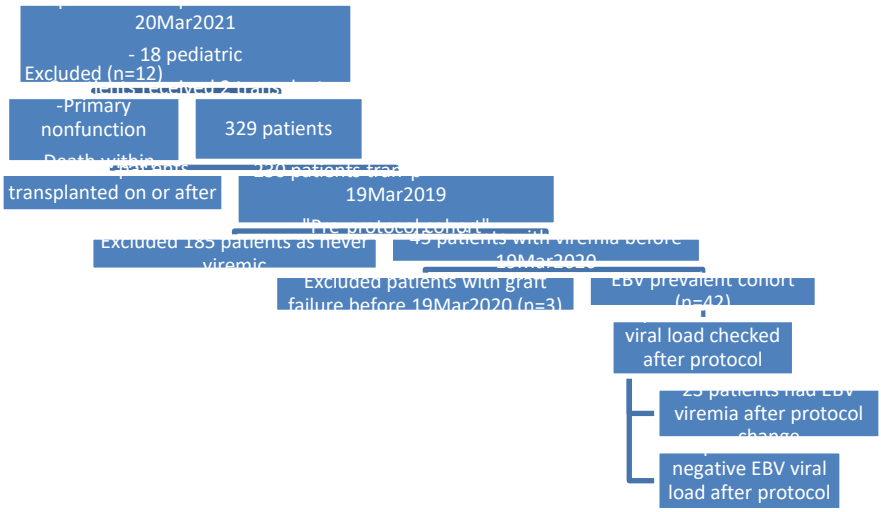

Figure 2a. Patient flow for the CMV incident cohort

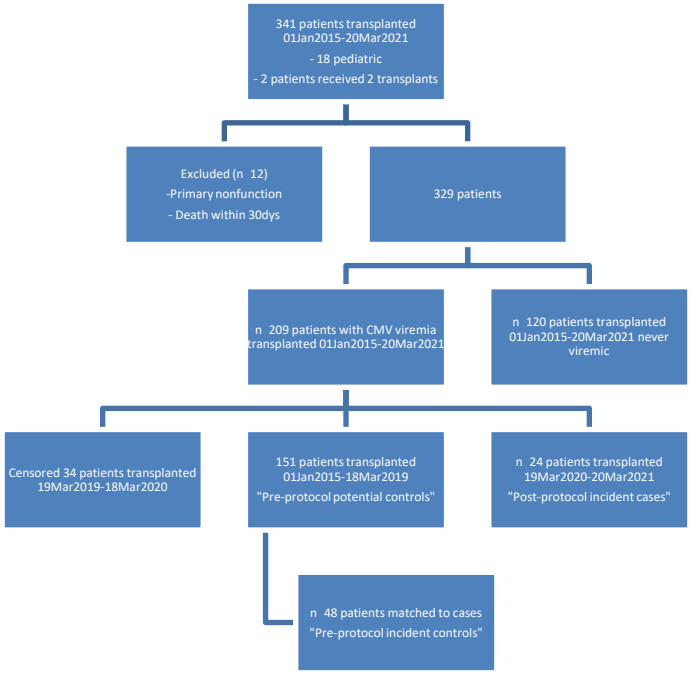

Table 3a. Baseline demographics of CMV viremic patients transplanted after 19Mar2020 (post-protocol incident cohort cases) (n=24) compared to patients transplanted 01Jan2015-19Mar2019 (matched pre-protocol controls) (n=48)

Baseline and perioperative variables	Controls n=48	Cases n=24	P-value
Age at transplant (yrs) (n=72)	53.5 (48, 64)	53.5 (47, 64)	0.9286
First transplant (%) (n=72)	43 (90%)	20 (83%)	0.4694
Male (%) (n=72)	33 (69%)	19 (79%)	0.4138
Caucasian (%) (n=72)	21 (44%)	7 (29%)	0.3074
Living donor (%) (n=72)	16 (33%)	7 (29%)	0.7935
Alloimmune risk category (%) (n=72) - Low - Intermediate - High	 11 (23%) 20 (42%) 17 (35%)	 8 (33%) 5 (21%) 11 (46%)	0.2329
Pre-transplant DSA (%) (n=72)	1 (2%)	1 (4%)	1.0000
cPRA (n=72)	0 (0, 16)	17 (0, 68)	0.1280
Induction (%) (n=72)	30 (63%)	18 (75%)	0.4268
Thymoglobulin (%) (n=72)	4 (8%)	1 (4%)	0.6588
CMV donor/recipient serology (n=71) - Neg/neg - Neg/pos - Pos/pos - Pos/neg	 3 (6%) 21 (44%) 23 (48%) 1 (2%)	 0 (0%) 12 (52%) 10 (43%) 1 (4%)	0.6100
High risk by serology (n=71)	4 (8%)	2 (8%)	1.0000

Table 3b. Outcomes of CMV viremic patients transplanted after 19Mar2020 (post-protocol incident cohort cases) (n=24) compared to patients transplanted 01Jan2015-19Mar2019 (matched pre-protocol controls) (n=48)

Outcome	Controls (n=48)	Cases (n=24)	P-value
Time to first viral load (dys)	37.5 (23, 113.3)	42.5 (31.8, 58.8)	0.9714
First viral load (copies/mL)	138 (138, 138)	138 (138, 334.5)	0.1009
Max viral load (copies/mL)	893 (138, 3458)	342 (138, 2085)	0.4086
Negative PCR achieved (%)	47 (98%)	23 (96%)	1.0000
Time to negative PCR (dys)	23 (13, 49)	46 (15, 58)	0.0555
Two consecutive negative PCRs at least 1wk apart achieved (%)	46 (96%)	23 (96%)	1.0000
Time to two consecutive negative PCRs at least 1wk apart (dys)	55 (35, 73.3)	66 (42, 90)	0.1613

Figure 2b. Patient flow for the CMV prevalent cohort

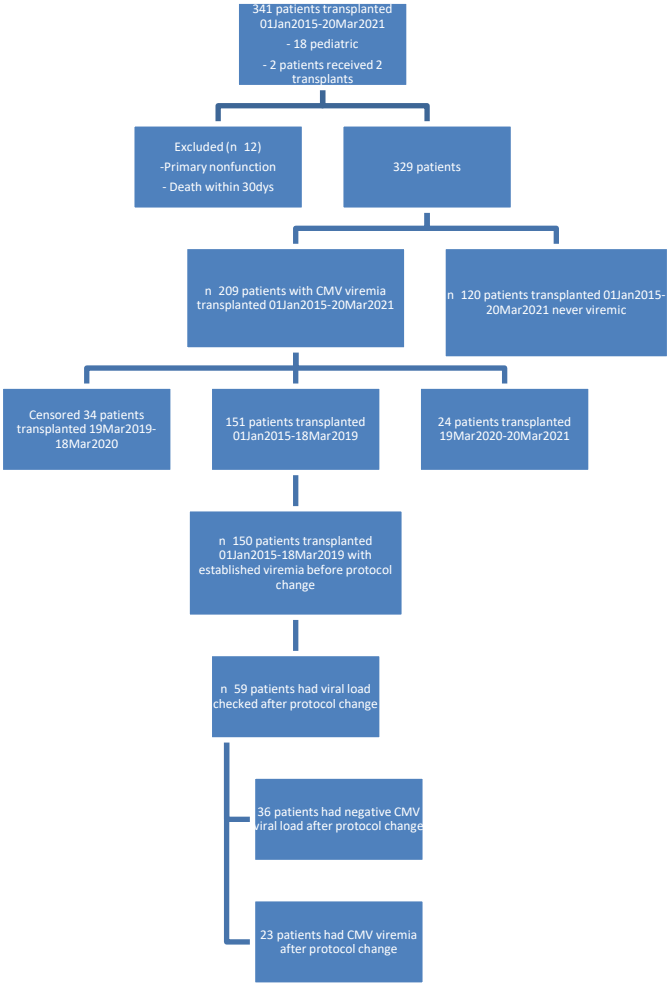

Figure 3a. Patient flow for the BKV incident cohort

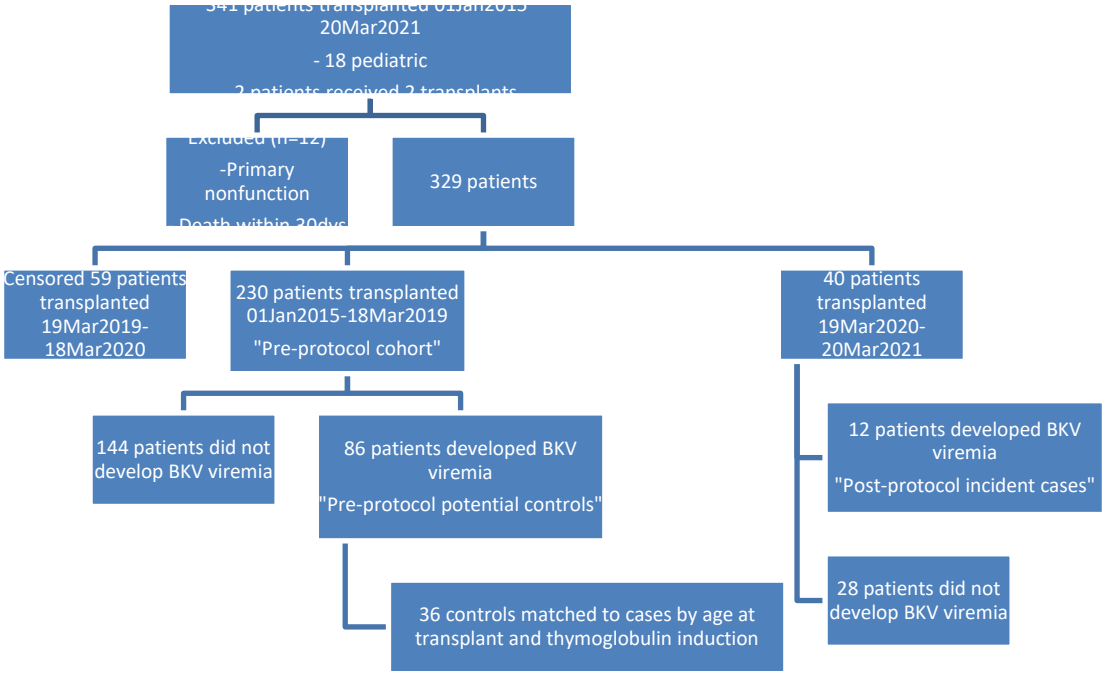

Table 4a. Baseline demographics of BKV viremic patients transplanted after 19Mar2020 (post-protocol incident cohort cases) (n=12) compared to patients transplanted 01Jan2015-19Mar2019 (matched pre-protocol controls) (n=36)

Baseline and perioperative variables	Controls (n=36)	Cases (n = 12)	P-value
Age at transplant (yrs)	51 (42, 64)	49 (42, 64)	0.8303
First transplant (%)	33 (92%)	9 (75%)	0.1558
Alloimmune risk category (%) - Low - Intermediate - High	 7 (20%) 12 (33%) 17 (49%)	 1 (100%) 0 0	0.2162
Pre-transplant DSA (%)	1 (3%)	0	1.0000
PRA	0	4	0.6254
Induction (%)	24 (67%)	10 (83%)	0.4651
Thymoglobulin (%)	3 (8%)	1 (8%)	1.0000
Preceding rejection (%)	8 (22%)	1 (8%)	0.4160
Preceding steroid bolus (%)	7 (19%)	1 (8%)	0.6593

Table 4b. Outcomes of BKV viremic patients transplanted after 19Mar2020 (post-protocol incident cohort cases) (n=12) compared to patients transplanted 01Jan2015-19Mar2019 (matched pre-protocol controls) (n=36)

Baseline and perioperative variables	Controls (n=36)	Cases (n = 12)	P-value
Time to first viral load (dys)	86 (58, 172)	76 (49, 83)	0.2731
First viral load (copies/mL)	1200 (500, 4675)	1650 (500, 11325)	0.6543
Max viral load (copies/mL)	18000 (2075, 135000)	78500 (6225, 152500)	0.4387
Negative PCR achieved (%)	31 (86%)	8 (67%)	0.1995
Time to first negative PCR (dys)	95 (58, 176)	87 (21, 275)	0.8893
Two consecutive negative PCRs at least 1wk apart achieved (%)	29 (81%)	6 (50%)	0.0610
Time to two consecutive negative PCRs at least 1wk apart (dys)	178 (76, 384)	143 (78, 179)	0.5399
BKVN			
Patients with BKVN (%)	30 (83%)	8 (67%)	0.2412
Time from first viral load to first BKVN (dys)	82 (15, 165)	54 (2, 119)	0.5940
Viral load pre-biopsy (copies/mL)	350000 (14375, 1562500)	130000 (120000, 740000)	1.0000
Serum creatinine at time of biopsy	230 (138, 368)	249 (166, 360)	0.9151

Figure 3b. Patient flow for the BKV prevalent cohort