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EDUCATIONAL REVIEW

Viral surveillance and subclinical viral infection in pediatric kidney transplantation

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Abstract The more potent immunosuppressive therapy that has successfully reduced the incidence of acute rejection and improved graft outcomes has also resulted in a higher incidence of viral complications. Sensitive molecular methods now allow for the detection of subclinical viral infection, which is increasingly recognized due to the adoption of routine post-transplant viral surveillance protocols. The goal of viral surveillance is the detection of subclinical viral infection that triggers an intervention; one that either prevents progression to viral disease or leads to early diagnosis of viral disease, which is associated with improved outcomes. Knowledge of the epidemiology and natural history of subclinical viral infection and viral disease, as well as patient-specific risk factors, is required to establish the optimal surveillance schedule which achieves the goal of early diagnosis. Evidence that detection of subclinical viral infection can impact viral disease is variable depending on the virus. This review will summarize the current data on the role of viral surveillance for BK virus (BKV), cytomegalovirus (CMV), and the Epstein–Barr virus (EBV) in the pediatric kidney transplant population.

Keywords Pediatric · Kidney transplant · Viral surveillance · Subclinical infection

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Introduction

Significant advances have been made in the outcomes of pediatric kidney transplant recipients. However, the more potent immunosuppressive therapy that successfully reduced the incidence of acute rejection has resulted in a higher incidence of viral complications [1]. In the pediatric kidney transplant population, infections have now replaced rejection as the leading cause of hospitalization [2].

Viral complications post-transplant are associated with different risks of morbidity and therefore need to be clearly defined. Subclinical viral infection is defined as a state of asymptomatic DNAemia characterized by the detectable presence of virus in blood without clinical symptoms or other laboratory abnormalities. Clinical viral disease is defined as viral replication with clinical features such as fever, leukopenia, and organ involvement [3–5]. Current anti-viral prophylaxis strategies do not appear to completely prevent subclinical viral infection. In addition, subclinical viral infections are not currently the target of systematic intervention or treatment with consensus guidelines, which do exist for viral disease [4, 5].

Viral surveillance

Viral surveillance refers to the routine monitoring of blood or urine for virus post-transplant. The schedule of viral surveillance varies by virus, patient characteristics (such as serostatus), and the individual transplant center. Some consensus recommendations exist but none specifically address the pediatric kidney transplant population. In general, more frequent monitoring is indicated early after transplant during the period of highest immunosuppression and then tapering off in frequency after 1 year post-transplant (See Tables 1 and 2). Additional screening is recommended for patients who

Table 1 Comparison of major group guidelines for BK virus (BKV) screening and intervention [6]

	2003 Polyoma-virus Associated Nephropathy Interdisciplinary Group [7]	2009 AST Infectious Diseases Group [8]	2009 KDIGO Transplant Work Group [4]
Screening	Urine screening, various techniques, every 3 months till month 24 (grade A-II) and annually thereafter till fifth year post-transplant (grade B-III) or with allograft dysfunction Biopsy if urine BK DNA>1×10^7, VP1 mRNA >6.5×10^5 or plasma DNA>1×10^4	Urine screening every 3 months in first 2 years then annually until fifth year post-transplant (grade II-B). If plasma screening is performed, then at monthly intervals. Biopsy if urine BK DNA>1×10^7, VP1 mRNA >6.5×10^5 or plasma DNA>1×10^4	Plasma BK nucleic acid testing monthly for first 3-6 months, then every 3 months till month 12, or if elevated serum creatinine or after treatment for acute rejection
Intervention	Various approaches discussed, none specifically endorsed	Reduce immunosuppression for presumptive BKVN (plasma BKV loads>1×10^4 for>3 weeks)	Reduce immunosuppression if plasma nucleic acid load persistently>1×10^4

AST American Society of Transplantation, *KDIGO* Kidney Disease Improving Global Outcomes

have had an increase in their immunosuppression such as following treatment for rejection.

The goal of viral surveillance is the detection of subclinical viral infection that triggers an intervention; one that either prevents progression to viral disease or leads to the early diagnosis of viral disease, which is associated with improved outcomes. Knowledge of the epidemiology and natural history of subclinical viral infection and viral disease, as well as patient-specific risk factors, is required to establish the optimal surveillance schedule that achieves the goal of early diagnosis. Evidence that detection of subclinical viral infection can impact viral disease is variable, depending on the virus. A recent retrospective study of pediatric kidney transplant recipients demonstrated an increased risk of full-blown viral disease in patients who had missed their routine viral surveillance [10].

BK virus

BK virus (BKV) was first isolated from the urine of a kidney transplant recipient in the 1970s [11], but it was not until the late 1990s that this virus emerged as a significant problem in kidney transplantation [12, 13]. BK virus is a part of the polyoma group of viruses and establishes latency in the uroepithelium. This propensity for the uroepithelium is responsible for the clinical manifestations of hemorrhagic cystitis in bone marrow transplant recipients and ureteral stenosis and allograft nephropathy in kidney transplant recipients.

Based on evidence that BK viremia and viruria appear prior to the onset of BK virus nephropathy, prospective screening for BK virus is currently recommended as part of routine post-transplant follow-up [4, 7]. Methods to identify BK virus include urine cytology for the identification of decoy cells, urine, and blood polymerase chain reaction (PCR). Whether to screen with PCR of urine or plasma has been an ongoing source of controversy. A negative urine PCR for BK virus has almost a 100 % negative predictive value [7]. Thus, some have favored testing urine and avoiding BK virus testing of blood on those patients with negative urine studies. However, the presence of BKV viruria, in the absence of elevated BK viremia, is not associated with an increased risk for BK virus nephropathy [7]. Hence, the use of urine screening requires follow-up testing of blood in the subset of patients with BK viruria. As a result, there is the potential for significant differences in the surveillance strategies used to follow renal transplant recipients. A survey of pediatric renal transplant centers in North America found that prospective screening is performed in the majority (91 %) of centers [14]. However, there was significant variability in the screening method of choice. Equal numbers of centers used either urine screening using cytology or PCR or plasma screening by PCR. In almost half of centers, a combination of both urine and plasma screening was used.

Much has recently been learned about the epidemiology of BKV nephropathy, which has guided the recommended surveillance schedule for BKV. Most BKV nephropathy occurs in the first 2 years after transplant with only 5 % of cases occurring between 2 and 5 years after transplant [7]. Accordingly, screening should be most intense early after transplant with decreasing frequency as patients are further post-transplant. Table 1 provides a comparison of major group guidelines for BKV screening and intervention. Specifically, Kidney Disease Improving Global Outcomes (KDIGO) recommends screening for BK virus monthly for the first 3–6 months after transplantation and then every 3 months until the end of the first post-transplant year [4]. The International Consensus Conference suggests continued annual screening for patients between 2 and 5 years after kidney transplantation [7]. In addition, screening is recommended in the setting of a decline in renal function from baseline. Further modification of screening algorithms can be made based on knowledge of

other risk factors for BKV nephropathy. The intensity of immunosuppression has been identified as one of the major risk factors for the development of BKV nephropathy [7]. As such, screening is recommended following an increase in the intensity of immunosuppression such as treatment for rejection. In addition, as novel immunosuppression protocols are developed and studied, more rigorous screening for viral complications such as BK virus would be prudent.

Reduction of immunosuppressive medication is the most common intervention in response to BK viremia. There is variability in the viral load which triggers intervention, although most agree that >10,000 copies/ml warrants response due to its positive predictive value for BKV nephropathy [4, 7]. The approach to immunosuppression reduction varies among centers, with varying levels of supporting evidence, and includes the following: (1) switching from tacrolimus to cyclosporin A (CSA) or sirolimus; (2) mycophenolate mofetil (MMF) to azathioprine or sirolimus or leflunomide; (3) decreasing tacrolimus (trough levels<6 ng/ml), MMF (dosing≤ 1 g/day), and CSA (trough levels 100-150 ng/ml); or (4) decreasing tacrolimus or MMF (maintain or switch to dual therapy with calcineurin inhibitor (CNI) and prednisone, sirolimus/prednisone, MMF/prednisone) [4]. Reduction of immunosuppression raises concerns about the unintended consequence of rejection. Several studies have reported successful preemptive intervention with no increase in rejection [15, 16].

Epstein–Barr virus

For Epstein–Barr (EBV) virus, the primary goal of viral surveillance is to prevent the development of post-transplant lymphoproliferative disorder (PTLD). The utility of viral surveillance again depends on knowledge of the natural history of subclinical viral infection and viral disease; in this case, PTLD. Prospective viral surveillance studies reveal that subclinical EBV infection occurs in 35-40 % of pediatric kidney transplant recipients [17, 18]. In a recent cohort study of adult kidney transplant recipients, 40 % had subclinical viremia [19]. Data reveal that EBV viremia often precedes the development of EBV disease and PTLD by 4-16 weeks [20, 21]. Thus, early identification of EBV viremia may allow for intervention that could prevent progression to EBV disease and PTLD. However, certainly not all patients with EBV viremia develop PTLD.

Awareness of risk factors associated with PTLD also guide viral surveillance strategies. Numerous risk factors have been identified including young age, Caucasian race, male gender, specific immunosuppressive medications, and type of organ transplanted [22–25]. However, primary EBV infection is considered to be the most important [22, 23, 26, 27]. Primary infection is defined as infection occurring in a seronegative recipient. Due to the seroepidemiology of primary EBV infection, pediatric patients are often EBV-seronegative, making them an exceptionally vulnerable population. Recent US data demonstrated that approximately 50 % of pediatric kidney transplants recipient were EBV-seronegative at the time of transplant compared to 10 % of adult kidney transplant recipients [28]. Surveillance strategies differ based on recipient serostatus. Table 2 provides a comparison of major group guidelines for EBV screening. KDIGO recommends the following post-transplant EBV schedule for high-risk D+/R- patients; once in the first week after transplant, at least monthly for the first 3-6 months, then at least every 3 months until the end of the first year with re-initiation of monitoring after treatment for acute rejection. While the D–/R– patient might be at decreased risk of developing EBV disease compared to D+/R–, they are still at increased risk relative to the R+ patient and therefore warrant close monitoring. Some centers may choose to measure EBV loads more frequently. Beyond the first year, selective monitoring, such as in those with persistently high viral loads or in those with higher than normal immunosuppression, may be performed based on center preferences. Some centers recommend continued monitoring for an indefinite period for all patients. For seropositive individuals, selective monitoring may be considered.

While there is agreement on the merits of EBV surveillance, debate remains about the optimal site of amplification of the virus (whole blood versus plasma versus PBMC) and the viral load that should prompt intervention [20, 29, 30]. In practice, the most important strategy is to follow the viral load in the same lab using the same type of sample consistently over time and to be careful to not compare viral loads from one lab to another.

Search for a threshold EBV viral load continues with no real consensus in part due to the lack of generalizability of PCR results from one center to another. There are conflicting results about whether a high or persistent EBV viral load is predictive of development of PTLD. Some have identified threshold levels of EBV viral load associated with the subsequent development of PTLD. McDiarmid et al. developed a protocol of EBV surveillance and pre-emptive therapy in pediatric liver transplant recipients that was successful in reducing the incidence of PTLD from 10 to 5 % [31]. It is important to remember that children, in particular, can develop a chronic high load carrier state without ever progressing to PTLD, which was confirmed recently in a prospective multicenter study of 106 pediatric kidney transplant recipients [32–38]. Nevertheless, the majority of reports indicate that higher EBV PCR values are associated with a greater risk for subsequent PTLD [39–41].

There is no universally accepted treatment for subclinical EBV infection post-transplant. Options include reduction of immunosuppression, antiviral therapy, IVIG, and monoclonal antibody therapy directed toward infected B lymphocytes [5, 21, 31, 42, 43]. Currently, the only consensus recommendation is for a reduction of immunosuppression in EBV-seronegative patients with an increasing EBV viral load [4].

Table 2 Summary of viral surveillance recommendations

	2009 KDIGO Transplant Work Group [4]	International Consensus Guidelines on the Management of CMV in Solid-Organ Transplantation [9]	AST recommendations for creening, monitoring, and reporting of infectious complications in immunosuppression trials in recipients of organ transplantation [5]	Seattle Children's Viral Surveillance Protocol	Washington University, St. Louis, Viral Surveillance Protocol
EBV	D+/R- Once in first week post-tx At least monthly for the first 3-6 m Every 3 months until end of first year Following treatment for acute rejection		***Seronegative recipients (including all children <1 year of age regardless of their pre-transplant EBV serostatus)*** ***First year:*** EBV viral load should be obtained at least once a month. Some centers may choose to measure EBV loads more frequently. ***Beyond 1st year:*** Selective monitoring, such as in those with persistently high viral loads or in those with higher than normal immunosuppression based on center preference. Some centers recommend continued monitoring for an indefinite period for all patients. ***Seropositive individuals (except for children <1 year of age)*** For new immunosuppressive agents, selective monitoring may be considered. EBV viral loads should be determined for all recipients with symptoms of PTLD	Donor and recipient should be screened by EBV serology prior to transplant. EBV PCR monthly for first year Every 3 months in the second year Resume monitoring after increase in immunosuppression for rejection	Donor and recipient should be screened by EBV serology prior to transplant. EBV PCR monthly in whole blood for first year
CMV	Monitoring of CMV viral load guided by prophylaxis strategy	Monitoring is recommended by some experts during prophylaxis due to the risk ofbreakthrough DNAemia. Frequency of monitoring should take into account prophylaxis, immunosuppressive regimen, and likelihood of compliance with the prophylactic regimen	Donor and recipient should be screened by CMV serology prior to transplant. Monitoring once a month (using a quantitative viral load assay) for the first year post-transplant is recommended. The duration and frequency may vary depending on the type of trial and the type of CMV prevention strategy	Donor and recipient should be screened by CMV serology prior to transplant. CMV PCR monthly for first year Every 3 months in second year Resume monitoring after increase in immunosuppression	Donor and recipient should be screened by CMV serology prior to transplant CMV PCR for clinical indication (all patients receive anti-viral prophylaxis)

EBV Epstein–Barr virus, *CMV* cytomegalovirus, *PTLD* post-transplant lymphoproliferative disorder, *PCR* polymerase chain reaction, *KDIGO* Kidney Disease Improving Global Outcomes, *AST* American Society of Transplantation

The utility of antiviral therapy to prevent PTLD is controversial with little evidence to support the role of acyclovir or ganciclovir in response to an elevated or rising EBV viral load without a concomitant reduction of immunosuppression. Preemptive use of rituximab in response to subclinical EBV infection began in the hematopoietic stem cell population and has recently been reported in the adult kidney transplant population [44, 45].

Cytomegalovirus

The goal of viral surveillance for cytomegalovirus (CMV) is to prevent CMV disease. However, the challenge of CMV post-transplant differs from BKV and EBV due to the availability of effective antiviral therapy. Published guidelines recommend regular CMV monitoring, however the duration and frequency may vary depending on the type of CMV prevention strategy, which is guided by the risk of primary infection [4, 5, 9]. Prevention of CMV infection can be accomplished with either (1) universal prophylaxis: the administration of anti-CMV therapy to all patients except seronegative recipients of a seronegative organ; or (2) preemptive therapy: viral monitoring and initiation of the treatment dose of antiviral medication when a certain positive threshold is reached. There is some controversy as to the optimal strategy, as both methods have advantages and disadvantages. Consensus guidelines from American Society of Transplantation (AST), KDIGO, and The Transplantation Society International CMV Consensus Group recommend universal prophylaxis for high-risk patients (seronegative recipients of seropositive organs or seropositive recipients of seropositive organs in the setting of anti-T-cell antibody immunosuppression), based on the available data suggesting better graft survival and clinical outcomes [4, 5, 9]. Approximately 65 % of pediatric kidney transplant recipients are CMV seronegative at the time of transplant, placing them in the high-risk category compared to 40 % of adult kidney transplant recipients [28]. Preemptive therapy has not been well studied in pediatrics.

Although several agents are available for prophylaxis, valganciclovir has revolutionized both CMV prevention and treatment [46]. It is a prodrug of ganciclovir and is approximately 60 % bioavailable which is tenfold more than ganciclovir. While the dosing of valganciclovir is well established in adults, the dosing in pediatric patients is somewhat more complex due to the dependence on metabolic activation, renal clearance, and variable absorption. Use of the valganciclovir dosing algorithm that is adjusted for body surface area (BSA) and creatinine clearance is recommended by the Transplantation Society International CMV Consensus Group [9]. Other centers have employed a weight-based approach [47]. Due to the challenges particularly in infants and young children, ganciclovir levels may be helpful to guide therapy. Leukopenia is a common side effect of valganciclovir therapy.

The duration of prophylaxis is an area of debate. Consensus recommendations guide the duration of therapy based on the serostatus of the donor and recipient [4, 9]. For CMV D+/R- patients, 3-6 months of prophylaxis with oral ganciclovir or valganciclovir is recommended. For CMV R+ patients, 3 months is recommended but 6 months should be considered if anti-lymphocyte induction is used. No prophylaxis is recommended in the CMV D-/R- patient. In addition, treatment of rejection with antilymphocyte antibodies in at risk recipients (D+/R-) should prompt re-initiation of prophylaxis or preemptive therapy for 1-3 months [4, 9, 48].

The timing and frequency of screening for CMV is largely center-specific and influenced by donor and recipient CMV serostatus. Published guidelines recommend regular monitoring using a quantitative viral load assay for the first year post-transplant, however the duration and frequency may vary depending on the type of CMV prevention strategy [4, 9]. The recent development of an international standard for CMV is promising as it will permit determination of appropriate standardized trigger points for intervention and allow comparison among sites.

Continued viral surveillance and close clinical follow-up is recommended even after prophylaxis is complete due to the risk of late-onset CMV disease, which is defined as disease occurring after prophylaxis has been discontinued. Late-onset CMV disease has been reported in 25-40 % of patients on universal prophylaxis and it can be associated with significant morbidity and mortality, underscoring the ability of anti-viral prophylaxis to delay but not prevent CMV [49, 50].

While antiviral prophylaxis has been effective in decreasing the incidence of clinically symptomatic CMV disease, subclinical infection remains relatively common. Limited data from prospective studies exist on the incidence and natural history of subclinical CMV infection in pediatric renal transplant recipients. Prospective viral surveillance studies have found subclinical CMV infection in 12-22 % of pediatric kidney transplant recipients [17, 18]. Therefore, subclinical CMV infection is not eliminated by the use of antiviral prophylaxis emphasizing the importance of viral surveillance.

Recommendations for treatment of asymptomatic infection in pediatric solid-organ transplant recipients have recently been made by the Transplantation Society International CMV Consensus Group [9]. In children younger than 5 years with asymptomatic DNAemia, initial treatment should be with intravenous ganciclovir (5 mg/kg every 12 h), although some experts recommend valganciclovir. In older children and adolescents, valganciclovir is recommended.

Subclinical viral infection and chronic allograft injury

As viral surveillance has become more common post-transplant, there has been an increased awareness of previously unrecognized adverse outcomes associated with subclinical infection. Emerging evidence suggests a role for subclinical viral infection in the pathogenesis of chronic allograft injury. The first line of evidence includes studies in which transplant recipients with subclinical viral infection developed higher rates of chronic allograft injury compared to those with no history of subclinical viral infection. Li et al. first reported an association between subclinical CMV and/or EBV viremia and decreased renal function in patients less than 5 years of age [17]. Subsequent work has demonstrated an association between subclinical CMV and EBV viremia and significant decreases in renal function with histologic evidence of moderate to severe chronic allograft injury at 2 years post-transplant [18]. In addition, there was a relationship observed between the level of viral exposure (viral load and number of viruses) and the degree of chronic allograft injury. Similar to the high risk for PTLD with primary EBV infection, primary subclinical infection was associated with a greater degree of allograft injury than reactivation subclinical infection. Chronic allograft injury has also been reported in recipients of heart, lung, and adult kidney transplants in associated with subclinical CMV infection [22, 51–55].

Unlike for viral disease, the mechanism of chronic allograft injury associated with subclinical viral infection is not well established. Whether direct viral cytopathic effects, indirect inflammatory effects, or a combination of multiple mechanisms leads to allograft injury remains a key question for future studies [56–59].

Cost

The optimal viral surveillance schedule is one that achieves the goal of early diagnosis but is also responsible in terms of cost. In a retrospective, single-center study comparing the cost-effectiveness of the various surveillance strategies, Laskin and Goebel demonstrated that surveillance for BK virus using plasma PCR was more cost-effective than urine PCR or cytology in pediatric renal transplant recipients [59]. A similar cost-effectiveness study was performed in adult transplant recipients [60]. Alternatives to the PCR-based surveillance strategies are needed in those areas of the world where this approach is cost prohibitive and therefore, unrealistic. The current challenge for all transplant programs is to determine the surveillance schedule that balances the cost of screening with the potential to prevent viral complications and the unintended consequences of intervention; rejection, graft loss.

Conclusions

Viral surveillance post-transplant has the potential to decrease post-transplant morbidity due to viral complications. Knowledge of the epidemiology and natural history of subclinical viral infection and viral disease, as well as patient-specific risk factors, is integral to determining the optimal surveillance schedule, which can allow for early diagnosis and trigger intervention. Some consensus recommendations exist but none specifically address the pediatric kidney transplant population who represent a vulnerable population due to their increased risk for primary infection. Ongoing work is needed to refine the transplant community's approach to viral surveillance balancing the potential benefits on graft and patient outcome, the cost of screening, and the optimal intervention that minimizes unintended consequences.

Questions (answers are provided following the reference list)

1. Screening for BK virus can be performed with which of the following methods:
   a. PCR of urine
   b. PCR of blood
   c. Urine for decoy cells
   d. All of the above.
2. The most important risk factor for PTLD is:
   a. Tacrolimus maintenance immunosuppression
   b. Primary EBV infection
   c. Caucasian race
   d. EBV reactivation
3. The most frequent viral surveillance for EBV is recommended for which patient population:
   a. EBV D-/R-
   b. All pediatric patients regardless of serostatus
   c. EBV D+/R-
   d. EBV D+/R+
4. Universal prophylaxis for CMV refers to:
   a. the administration of anti-CMV therapy to all patients except seronegative recipients of a seronegative organ
   b. viral monitoring and initiation of the treatment dose of anti-viral medication when a certain positive threshold is reached
   c. CMV IVIG
   d. the administration of anti-CMV therapy to all seropositive recipients

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Answers

1. d
2. b
3. c
4. a