American Society of Transplantation Recommendations for Screening, Monitoring and Reporting of Infectious Complications in Immunosuppression Trials in Recipients of Organ Transplantation

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In recent years, major progress has been made in the development, investigation and clinical application of novel immunosuppressive drug therapies to prevent acute rejection. Critical to the ultimate clinical application of new drug therapies is the ongoing performance of large multi-center clinical trials. However, there has been a paucity of infectious disease monitoring built into these protocols. Many protocols allow for passive surveillance and reporting of infections as adverse events. Even when protocols stipulate monitoring for a particular type of infection, different sites within a single multi-center study may have varying definitions for infection and use diverse testing assays. Accordingly, accurate assessment of the risk of infectious disease complications associated with a new immunosuppressive regimen is often impossible. Given that infectious complications are a major source of morbidity and mortality in transplant recipients, the assessment of the magnitude of risk of infection associated with a given immunosuppressive strategy may be as important as the assessment of rejection.

Purpose of Recommendations

For the above reasons, screening, monitoring and reporting recommendations for common transplant-associated infections were developed for use in clinical trials evaluating immunosuppressive strategies. These recommendations have two major goals: (i) to provide clinically relevant definitions for tracking infectious complications occurring in participants in immunosuppressive trials and (ii) where appropriate, to recommend specific laboratory monitoring and surveillance methods. It is hoped that the adoption of a common set of definitions and laboratory monitoring strategies will allow for accurate assessment of infectious risks associated with novel immunosuppressive strategies. It should be emphasized that when laboratory monitoring is recommended for a specific pathogen, its purpose is to allow accurate comparison of infectious risk in patients receiving differing immunosuppressive regimens as part of a clinical trial. Its purpose is not for management or prevention of specific pathogens and it does not supplant clinical monitoring strategies that may already be in place at participating centers. In fact it is anticipated that for large multi-center trials, the recommended laboratory protocols may not be done in ‘real time’ but rather through batching of specimens to facilitate monitoring.

Key words: Clinical trials, immunosuppression, infectious diseases

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Infectious Disease Monitoring Recommendations

Table 1: Summary of recommended laboratory monitoring strategies for trials evaluating new immunosuppressive regimens in transplant patients

<table>
<thead>
<tr>
<th>Organism</th>
<th>Recommended monitoring</th>
<th>Recommended test</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytomegalovirus</td>
<td>Yes: Minimum of monthly</td>
<td>Quantitative viral load assessment from blood</td>
<td>Duration may depend on prophylaxis regimen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Quantitative viral load assessment from blood</td>
<td>High risk populations include pediatric EBV negative recipients and EBV D+/R−</td>
</tr>
<tr>
<td>Epstein-Barr virus</td>
<td>Consider monitoring only in certain high risk populations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other herpes viruses</td>
<td>No routine monitoring</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BK virus</td>
<td>Yes: monthly × 6 months, then months 9 and 12 (kidney trials only)</td>
<td>Quantitative viral load assessment from blood and/or urine</td>
<td>Monitoring not needed in nonrenal transplant recipients</td>
</tr>
<tr>
<td>Hepatitis C virus</td>
<td>Yes: in HCV positive liver and HCV positive nonliver transplants</td>
<td>Quantitative viral load from blood and surveillance biopsies of liver</td>
<td></td>
</tr>
<tr>
<td>Fungal infections</td>
<td>No routine lab monitoring</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial infections</td>
<td>No routine lab monitoring</td>
<td></td>
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</tbody>
</table>

Some trials may be sufficiently large to detect statistically significant differences in infectious risk. However, others may be underpowered to detect such differences, depending on the number of patients enrolled and the incidence of a particular infectious complication. Caution should be exercised in the interpretation of nonsignificant trends, especially in smaller trials. At best, such trends may serve as a potential warning, thereby warranting further evaluation and study.

SPECIFIC PATHOGENS

Definitions of infection and disease along with detailed recommendations for monitoring are provided for some of the more common infectious complications that are observed in recipients of solid organ transplantation. In addition, monitoring for specific protocols must be adapted for any unique risks of a specific study population including endemic pathogens such as tuberculosis or parasites. A summary of laboratory monitoring recommendations is provided in Table 1. Where specific virologic monitoring is recommended, it is preferable to use a core laboratory performing a single assay to ensure accuracy and consistency across centers or for each center to use the same commercially available, standardized molecular quantitative assay to allow comparison across centers.

VIRAL INFECTIONS

Cytomegalovirus

Background

Cytomegalovirus (CMV) infection in the transplant recipient remains a significant cause of morbidity and mortality and may have both direct and indirect effects. Risk factors for CMV infection include the intensity of immunosuppression and the serological status of donor and recipient (highest risk Donor (D)+/Recipient (R)− (1–3). The effects of investigational and more potent immunosuppressive regimens on the spectrum of CMV disease in organ transplant recipients are largely unknown. Accordingly, it is critical that clinical trials evaluating novel immunosuppressive regimens be cognizant of CMV as a potential adverse event and monitor for it in a consistent fashion.

Various strategies or combinations of strategies are used to combat CMV infection after transplantation including pre-emptive therapy and universal prophylaxis (1–3). Since many different approaches to CMV prevention exist, it is important to control for this variable in clinical trials comparing immunosuppressive regimens. Establishment of a consistent strategy of CMV prevention that applies to all study centers before a trial begins is strongly encouraged. In the absence of a single CMV prevention strategy across multiple sites, the type of strategy should be clearly documented for each center.

Definitions

We propose the following definitions for use in trials of immunosuppression agents:

**CMV active infection**: Replicative infection can be diagnosed by growing the virus in vitro, finding evidence of viral infection by intra-cytoplasmic or intra-nuclear inclusions or by antibody-based staining techniques for CMV in histopathologic sections or finding evidence of replication using nucleic acid based assays or antigenemia studies (4,5).

**CMV disease**: CMV disease is defined by evidence of CMV infection with attributable symptoms. CMV disease can be subclassified into CMV viral syndrome or tissue invasive disease. Proposed definitions are listed in Table 2 and have been adapted from several sources including the AST Infectious Diseases Guidelines and Canadian Society of Transplantation consensus conference on CMV (1,2,6).

Laboratory monitoring

We recommend including specific laboratory monitoring for CMV as part of all immunosuppression trials. The duration and frequency of monitoring should take into account the time period in which CMV viremia is most likely to
### Table 2: Definitions of CMV disease in solid organ transplant recipients

<table>
<thead>
<tr>
<th>Disease type</th>
<th>Probable</th>
<th>Definite</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMV syndrome</td>
<td>One or more of the following:</td>
<td>Clinical and laboratory findings as in “probable” case and no other cause of symptoms/signs identified</td>
</tr>
<tr>
<td></td>
<td>1. Fever &gt;38°C for at least 2 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2. New or increased malaise</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3. Leukopenia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4. ≥5% atypical lymphocytes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5. Thrombocytopenia</td>
<td></td>
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<tr>
<td></td>
<td>6. Elevation of hepatic transaminases (ALT or AST) to 2 × upper limit of normal (applicable to nonliver transplant recipients) plus evidence of CMV in blood by viral culture, antigenemia or a DNA/RNA-based assay</td>
<td></td>
</tr>
<tr>
<td>Pneumonia1</td>
<td>Signs and/or symptoms of pulmonary disease in the absence of other documented cause plus evidence of CMV in blood</td>
<td>Signs and/or symptoms of pulmonary disease plus detection of CMV in lung tissue by culture, immunohistochemical analysis or in situ hybridization⁴ with or without evidence of CMV in blood or BAL fluid by viral culture, antigenemia (BAL) or a DNA/RNA-based assay</td>
</tr>
<tr>
<td></td>
<td>and/or³ bronchoalveolar lavage (BAL) fluid by viral culture, antigenemia or a DNA/RNA-based assay</td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal disease</td>
<td>Symptoms of upper or lower gastrointestinal disease plus macroscopic mucosal lesions on endoscopy plus evidence of CMV in blood or biopsy tissue by viral culture, antigenemia or an RNA/DNA-based assay</td>
<td>Symptoms or signs of upper or lower gastrointestinal disease plus detection of CMV in gastrointestinal tissue by culture, immunohistochemical analysis or in situ hybridization⁴</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>Elevation of bilirubin and/or hepatic enzymes in the absence of other documented cause of hepatitis² plus evidence of CMV in blood by anti-genemia or a DNR/RNA-based assay</td>
<td>Elevation of bilirubin and/or hepatic enzymes plus detection of CMV in liver tissue by culture, immunohistochemical analysis or in situ hybridization⁴</td>
</tr>
<tr>
<td>CNS disease</td>
<td>CNS symptoms in the absence of other documented cause plus evidence for CMV in CSF samples by viral culture or DNA-based assay</td>
<td>CNS symptoms plus detection of CMV in CNS tissue by culture, immunohistochemical analysis or in situ hybridization⁴</td>
</tr>
<tr>
<td>Retinitis</td>
<td>Not applicable</td>
<td>Lesions typical of CMV retinitis must be confirmed by an ophthalmologist</td>
</tr>
<tr>
<td>Other tissue invasive disease</td>
<td>Evidence of organ dysfunction in the absence of other documented cause² plus evidence of CMV in blood by viral culture, antigenemia or DNA/RNA-based assay</td>
<td>Symptoms/signs of organ dysfunction plus detection of CMV in affected tissue by culture, immunohistochemical analysis or in situ hybridization⁴</td>
</tr>
</tbody>
</table>

1. Superinfection or coinfection with other pathogens may occur and should be noted when present.
2. If affected organ is the allograft, acute rejection must be excluded as a cause for the clinical symptoms.
3. The detection of CMV in both BAL and peripheral blood strengthens the evidence for probable CMV pneumonitis.
4. Although, immunohistochemistry and in situ hybridization techniques are more sensitive for the detection of CMV-infected cells than morphologic examination, the presence of typical cytomegalovirus inclusions should be considered evidence of definite disease.

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occur. Classically, in the nonprophylaxed patient, CMV occurs most commonly between the 1st and 4th month after transplant. However, because prophylaxis can delay the onset of CMV, we recommend that the frequency of monitoring should be at least monthly for 1-year post-transplant in all patients at risk (all except CMV donor negative/recipient negative combinations). However, the selection of times for viral load monitoring may vary depending upon the specifics of the clinical trial and the types of CMV prevention strategies that are in place. For example, depending on the particular immunosuppression regimen being evaluated and the type of CMV prophylaxis, a study steering
committee may elect not to monitor for CMV while patients are on effective CMV prophylaxis, if the risk of breakthrough viremia is known to be low.

While a number of potential laboratory assays are available for CMV, a quantitative molecular assay (viral load test) is recommended for monitoring. As yet, no specific quantitative molecular assay has been shown to be definitively superior to the others and no standardized method has been adopted by either commercial laboratories or university centers. However, within a given study, the same testing method should be used across all centers. This can be done by using a standardized commercially available assay or performing all testing at a core facility. Consideration should also be given to obtaining samples for anti-viral resistance testing for patients who develop CMV disease, especially if anti-viral prophylaxis is used. It will be important to document the relationship between novel immunosuppressive strategies and the likelihood of emergence of anti-viral resistance. Genotypic resistance testing should be performed looking for UL97 and UL54 mutations; phenotypic testing may also be performed, but is more time-consuming, expensive and labor-intensive (7).

**Summary of recommendations for CMV screening, monitoring and reporting**

- **Screening**
  - Donor and recipient should be screened by CMV serology prior to transplant.

- **Monitoring**
  - Immunosuppression trials should include a laboratory monitoring strategy for CMV for all risk groups except D+/R– patients.
  - Monitoring once a month (using a quantitative viral load assay) for the first year post-transplant is recommended. However, the duration and frequency may vary depending on the type of trial and the type of CMV prevention strategy.

- **Reporting**
  - Standard definitions for CMV infection and disease should be agreed upon prior to initiation of the study (see Table 2 and text).

**Epstein-Barr Virus**

**Background**

Epstein-Barr virus (EBV), a ubiquitous herpes virus, infects humans with 40–90% seroprevalence by adolescence depending on socioeconomic status. The oncogenic potential of EBV relates to its ability to transform and immortalize B-lymphocytes, leading to the potential for uncontrolled proliferation of these transformed cells—particularly in immunodeficient individuals. The clinical syndromes that result from the uncontrolled proliferation of lymphocytes constitute post-transplant lymphoproliferative disorders (PTLD) (8–10).

Primary EBV infection in seronegative transplant recipients has been associated with an increased risk of developing PTLD (8,11). The highest risk occurs in seronegative recipients who receive an organ from a seropositive donor (D+/R–). In contrast, EBV-associated PTLD occurs frequently in patients who are EBV seropositive prior to transplant. The use of specific anti-T-cell therapies (e.g. OKT3) is also associated with an increased risk of developing PTLD (12–14). The use of more potent immunosuppressive agents may carry an increased risk for PTLD (13). For these reasons, trials of immunosuppressive regimens should prospectively monitor for the presence of EBV disease, particularly in seronegative recipients.

**Definitions**

**EBV infection:** The presence of past EBV infection is defined by the demonstration of positive serology in absence of passive antibody. Active, asymptomatic EBV infection is defined by the presence of a detectable EBV viral load as measured by a nucleic acid amplification assay. Uncommonly, asymptomatic infection may also be identified in lymphoid rich histopathologic specimens.

**EBV disease:** EBV disease is defined by the presence of active EBV infection with symptoms or signs attributable to the virus. The spectrum of clinical manifestations of EBV in transplant recipients includes nonspecific viral syndrome, mononucleosis, lymphoproliferative disorders and malignant lymphomas.

**Post-transplant lymphoproliferative disorder:** The gold standard for diagnosing PTLD is histopathologic examination (8,9). Tissue samples should be interpreted by a hematopathologist or pathologist who is familiar with the histopathologic features of PTLD, using a standardized classification system (9,10). PTLD is characterized by effacement and destruction of underlying architecture by a lymphoproliferative process (10). In addition to the morphologic classification, PTLD tissues should be examined for the presence of EBV by in situ hybridization. Further characterization may include the cell of origin (B, T, null, mixed), B-cell subpopulation (e.g. CD20). The evaluation of patients with PTLD should also include radiographic evaluation to document the extent of disease (e.g. CT scans) (8,15). Some experts recommend performance of bone marrow biopsies.

**Laboratory monitoring**

Serology for previous EBV infection should be obtained prior to transplantation for all recipients and donors. After transplantation, serology may be less reliable. Accordingly, nucleic acid based assays such as polymerized chain reaction (PCR) are valuable in identifying the presence of active EBV infection after transplantation (8,15–19).
**Recommended virologic evaluation**

There is no established consensus regarding the most appropriate schedule for laboratory monitoring. The following serves as a guide in order to facilitate a uniform approach in settings where new immunosuppressive agents are being evaluated.

(i) Pre-transplantation

EBV serology should be obtained prior to transplantation on all recipients. Donor serology is important in establishing the risk profiles (i.e. D+/R−) for EBV seronegative recipients. While some experts recommend performance of only the EBV VCA IgG and EBNA antibodies, others recommend obtaining a full serologic panel. Children less than 1 year of age are presumed to be seronegative, but may have positive results due to the presence of maternal antibodies.

(ii) Post-transplant EBV surveillance

**Seronegative recipients (including all children < 1 year of age regardless of their pre-transplant EBV serostatus)**

**First 1 year:**

- EBV viral load should be obtained at least once a month for EBV R− patients. 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 1st 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.

**Seropositive individuals (except for children < 1 year of age)**

These individuals are at less risk of developing PTLD compared with their R− counterparts. For new immunosuppressive agents, with undetected infectious diseases risk potential, selective monitoring may be considered, especially in pediatric trials. EBV viral loads should be determined for all recipients with symptoms of PTLD.

**Laboratory methods for measuring EBV load**

EBV viral load testing suffers from lack of standardization and the optimum assay technique and sampling sites are not well established. There is debate regarding the use of lymphocytes, whole blood or plasma for such assays. While no firm recommendations can be made regarding the preferred approach, for immunosuppression trials it is preferable to have assays performed centrally for purposes of uniformity.

**Other clinical and laboratory evaluations:** In addition to viral load testing, additional tests (e.g. imaging and biopsies) may be required for investigation of sustained elevations of viral load and/or signs/symptoms of EBV disease. In addition, consideration should be given to having an experienced central pathologist review of the histopathology of tissue samples.

**Summary of recommendations for EBV screening, monitoring and reporting**

- **Screening**
  - Donor and recipient should be screened by EBV serology prior to transplant.

- **Monitoring**
  - Immunosuppression trials should include a laboratory monitoring strategy for select populations such as EBV seronegative patients and pediatric trials.
  - The frequency of monitoring should be a minimum of once a month for the first year post-transplant. However, the duration and frequency may vary depending on the type of trial and the type of EBV prevention strategy.

- **Reporting**
  - Standard definitions for EBV infection and disease should be agreed upon prior to initiation of the study (see text).

**Other Herpes Viruses**

**Varicella Zoster Virus (VZV), Herpes Simplex Virus (HSV), Human Herpes Virus (HHV)-6, -7, and -8 (Kaposi’s sarcoma-associated herpes virus, KSHV)**

**Background:** Infections due to these ‘other herpes viruses’ occur commonly and may be due to reactivation of latent virus or primary infection. Herpes simplex virus (HSV) usually causes orolabial disease and genital or perianal disease (20–23). Invasive or disseminated disease may occur and includes esophagitis, hepatitis and pneumonitis (20–23). Varicella zoster virus (VZV) infection may occur due to reactivation in seropositive patient and result in local or disseminated zoster. Seronegative recipients (often children) are at risk for primary infection if exposed to VZV which can result in severe and even fatal infections (20,21). Visceral complications of VZV infection include pneumonitis, hepatitis and encephalitis (21). HHV-8 is the etiologic agent of Kaposi’s sarcoma (KS), primary effusion lymphoma and multicentric Castleman’s disease (24). The incidence of KS after transplant ranges from about 0.5% to 5% depending on the geographical location and other epidemiologic factors (25).

HHV-6 and HHV-7 usually infect humans within the first few years of life. Seroprevalence rates in adults are over 90% (26,27). In adult transplant recipients, most episodes of viremia are likely due to reactivation of endogenous latent virus. Rates of infection following transplant are between 31–55% and 0–46% for HHV-6 infection (usually variant B).
and HHV-7, respectively (20). Viremia may have direct or indirect clinical consequences. Symptomatic disease has primarily been described with HHV-6 and includes febrile illness, pneumonitis, hepatitis, encephalitis and bone marrow suppression (28). Both viruses may have immunomodulatory effects and appear to be risk factors for CMV disease and other opportunistic infections and may also have effects on graft function (26,27).

At present, active surveillance for this group of viruses is not felt to be required. However, standard definitions may assist with documenting infections if they occur.

Clinical definitions
The following definitions are proposed for use in clinical trials of immunosuppressive agents:

HSV 1 and 2

Asymptomatic shedding: defined by detection of HSV (e.g. by culture, DNA/RNA based assay, antigen detection) from a clinical specimen in the absence of any clinical symptoms.

Oroabial or genital HSV: defined by presence of vesicular type lesions in orolabial or genital areas. While virological confirmation is preferred, it is not essential to confirm a clinical diagnosis.

Tissue-invasive HSV: defined by presence of organ dysfunction in the absence of other documented cause with detection of HSV in biopsy tissue by culture, DNA/RNA based assay, immunohistochemical analysis or in situ hybridization. For HSV pneumonitis histopathologic evidence of HSV inclusions in lung tissue is necessary. A positive viral culture from a bronchoalveolar lavage sample is insufficient evidence as it may reflect shedding from the oropharynx.

Varicella zoster virus

Cutaneous VZV infection:

Probable: The presence of crops of vesicular lesions on an erythematous base is suggestive of the clinical diagnosis of cutaneous VZV and can be classified as probable. This may be primary infection (in a previously naïve host) or reactivation. Dermatomal distribution is sufficient to diagnose probable reactivation disease of VZV (zoster, shingles). Lesions in transplant patients may be atypical and difficult to diagnose.

Definite: Definite VZV infection is defined by the presence of typical lesions with isolation of VZV, demonstration of VZV antigen by direct fluorescent antibody (DFA) or by DNA/RNA-based tests from a lesion. Tissue invasive VZV infection is defined by the presence of organ dysfunction in the absence of another documented cause in association with detection of VZV in biopsy tissue by culture, DNA/RNA-based assay, immunohistochemical analysis or in situ hybridization.

HHV-6 or -7 infections:

Infection with these viruses is defined by evidence of replicative virus regardless of the presence of symptoms. Diagnostic tests must be able to distinguish active viral infection from latent infection. For example, a positive viral culture from any site, or detection of nucleic acid in a cell-free sample (e.g. plasma) would constitute evidence of active infection. An increasing viral load above baseline or a very high viral load is indicative of an active infection.

HHV-6 or -7 disease:

Febrile viral syndrome: This syndrome is defined by the presence of fever, with or without cytopenia, with evidence of HHV-6 or -7 in blood sample by culture or nucleic acid detection test. These viruses may serve as cofactors in patients with CMV viral syndrome. Febrile patients with coexistent CMV detection should be classified as dual infections.

Tissue invasive disease: This has generally been well documented only with HHV-6 and is defined by the presence of organ dysfunction in the absence of other documented cause with detection of HHV-6 in biopsy tissue or cerebral spinal fluid (CSF) by culture, DNA/RNA-based assay, immunohistochemical analysis or in situ hybridization. If the organ involved is the allograft, rejection should be excluded by evaluation of histology.

HHV-8:

The diagnosis of cutaneous or visceral Kaposi’s sarcoma should be based on biopsy showing histopathology consistent with KS (24). Demonstration of HHV-8 in the lesion by immunohistochemistry or in situ hybridization is also diagnostic and PCR of blood is supportive of the diagnosis.

Laboratory monitoring

Based on current data, routine laboratory monitoring of these herpes viruses is not recommended in the setting of an immunosuppression trial unless the recipients may be at significantly increased risk for specific diseases (e.g. KS in HIV+ subjects). Laboratory testing should be done for specific symptoms or clinical signs. Pre-transplant serology for HSV and VZV is recommended.

Summary of recommendations for screening, monitoring, and reporting for other herpes viruses

- Screening
  - Pre-transplant recipient serology should be obtained for HSV1, HSV2 and VZV.
- Monitoring
  - Laboratory monitoring is not routinely recommended.
- Reporting
  - HSV disease should be reported as orolabial, genital or tissue invasive (e.g. esophagitis, pneumonitis) and relationship to timing of anti-viral prophylaxis should be included.
  - VZV disease should be reported as primary or reactivation and the characteristics of disease should be identified (e.g. dermatomal, disseminated, visceral...
involvement) and relationship to timing of anti-viral pophylaxis should be noted.

- Any occurrence of KS or confirmed HHV-6 or HHV-7-related disease should be reported.

**BK Virus**

**Background**

BK, JC and SV40 viruses are closely related members of the polyomavirus family. Serological studies have shown that BK and JC virus infections occur worldwide in childhood. BK virus associated renal allograft nephropathy (BKVAN) has recently emerged as a major cause of renal allograft dysfunction (29–31). BKVAN is diagnosed on a kidney biopsy in patients presenting with a rise in serum creatinine in the absence of obvious preceding or concurrent symptoms. BKVAN is presumed to result from reactivation of virus latent in the renal tubules. However, affected children may have a primary infection with BK virus (32). Although BK virus disease is primarily limited to the allograft and ureters of kidney transplant recipients, native kidney involvement has occurred in recipients of nonrenal solid organs (33). JC virus and/or SV40 virus may also be detectable in kidney biopsy samples in conjunction with BK virus, although the contribution of these to the disease process is unclear. The rapid emergence of BKVAN as an important cause of renal allograft loss is likely related to the introduction of modern more potent immunosuppressive regimens. Therefore, it is important that a prospective evaluation of BK virus replication be included in any trial assessing new immunosuppressive strategies in renal transplant recipients.

**Clinical definitions**

No standard definitions exist for classifying infections due to BK virus. The most widely used definitions use results of kidney biopsies to document the presence of BKVAN (34). The following is proposed for use in clinical trials of immunosuppressive agents:

**BK virus infection (replicative infection) can be defined as:**

1. **Quantitative BK viral DNA load, in blood or urine above the detection threshold for the given laboratory’s assay.** BK infection should be classified as either BK viruria or BK viremia or both. While the presence of decoy cells in urine on cytology is suggestive of BK infection, confirmation with a specific test (PCR) is required.

   **Or**

2. **Evidence of BK virus nephropathy (as below)**

Proven BK virus nephropathy is defined by the presence of:

- Renal biopsy associated with (a) an acute tubular necrosis-like picture or (b) interstitial nephritis mimicking acute rejection or (c) chronic allograft nephropathy with confirmation of the presence of BK virus by electron microscopy, immunohistochemistry or **in situ** hybridization for BK virus. Although intra-nuclear viral inclusions are usually seen, their presence is not mandatory for a diagnosis of BKVAN.

In clinical practice, presumptive BKVAN is sometimes diagnosed in the presence of renal allograft dysfunction and a positive BKV DNA PCR result from blood in a patient with no viral inclusions at light microscopy and negative immunohistochemistry/**in situ** hybridization (based on the premise is that a biopsy can miss focal disease) (34). However, for reporting in the context of a clinical trial, these patients should be classified as BK virus infection (i.e. BK viremia rather than BKVAN).

**Laboratory monitoring**

Routine monitoring for BK virus is recommended for all kidney transplant immunosuppression trials. BKV monitoring strategies are still in development but a clinical trial should use a quantitative nucleic acid based viral load assay of urine and/or blood (29–31). Detectable virus in the blood is more predictive of BKVAN than viruria alone (34–36). While urine cytology (for decoy cells) is commonly used for early detection of BK replication, accurate comparison of infection risk in an immunosuppression trial is best determined by a quantitative objective test such as viral load. A proposed monitoring strategy assessing plasma, whole blood or urine BK viral loads at regular intervals post-transplant is outlined below. Laboratory evaluation should also be done for any unexplained rise in serum creatinine and should include a urinary and peripheral blood BK PCR and biopsy tissue immuno-staining or **in situ** hybridization for BK virus. Measurement of BK viral load does not preclude use of urine decoy cell monitoring as per center specific protocols.

**Summary of recommendations for screening, monitoring and reporting for polyomavirus**

- **Screening**
  - Pre-transplant donor and recipient serology not routinely required.
- **Monitoring**
  - For kidney transplant trials only, monitor plasma or whole blood or urinary BK viral load at a minimum of once a month for 6 months, then at months 9 and 12 post-transplant. If follow-up in a clinical trial is longer than 1 year, monitoring for BK virus is suggested every 3 months until 2 years, and then annually thereafter.
- **Reporting**
  - Reporting should include:
    - BK infection with viral replication as documented by PCR on urine or blood (specified as either BK viruria, viremia or both).
Infectious Disease Monitoring Recommendations

■ BK infection with nephropathy (BKVAN) as documented by allograft biopsy with positive immunohistochemistry, electron microscopy or in situ hybridization for BK virus.

Hepatitis C Virus

Background
End stage liver disease due to hepatitis C virus (HCV) is now the leading indication for liver transplantation worldwide. The course of HCV reinfection is accelerated in liver transplant recipients compared to the immunocompetent host. Nearly 90% of HCV-positive patients develop chronic allograft hepatitis at 5 years post-transplant and 20–30% develop allograft cirrhosis (37–39). The intensity and type of immunosuppression may influence the progression of post-transplant HCV disease (40,41). Monitoring for HCV should be built into liver transplant trials that include HCV positive patients.

Patients undergoing transplantation of other organs (non-liver transplants) may also have coexisting chronic HCV infection with varying stages of liver disease prior to transplantation. HCV progression may be accelerated in these individuals as well (42).

Diagnostic methods for HCV include serology, nucleic acid based testing, liver biopsy and genotyping. The enzyme immunoassay (EIA) is used for detection of antibodies to recombinant HCV peptides. HCV RNA can be detected by PCR or branched-chain DNA assay. Quantitative PCR or viral load measurement may be a useful marker to follow in immunosuppression transplant trials in patients with HCV. HCV genotyping may be helpful in predicting response to therapy, but its effect on post-transplant outcomes remains controversial (43). Histopathology is the gold standard for assessing the severity and prognosis of HCV disease and is used to guide anti-viral therapy and to differentiate allograft rejection from recurrent HCV disease.

Clinical definitions
Recurrent hepatitis C (liver transplant trials):
HCV reinfection after transplant is nearly universal. For the purposes of immunosuppression trials, recurrent disease can be classified as follows:

Histological recurrence: Evidence of biopsy-proven recurrence of hepatitis C. The degree of necroinflammatory activity and fibrosis should be graded on each biopsy using a standard grading system (examples include the Batts and Ludwig system, METAVIR and Knodell Histological Activity Index (HAI)) (44). Centers participating in multi-center immunosuppression trials should agree on a uniform grading system for the purposes of reporting outcomes in the trials prior to starting the trial.

Severe recurrence can be defined as recurrence of HCV resulting in graft loss, death or patients with cholestatic hepatitis.

Laboratory monitoring
Liver transplant trials: Routine laboratory monitoring is recommended in all liver transplant trials enrolling HCV-positive patients. Monitoring recommendations include viral load testing and routine surveillance biopsies as outlined below.

Nonliver transplant trials: Patients with chronic HCV infection who receive other organs (e.g. kidney transplant recipient with HCV) may sometimes be included in immunosuppression trials. If so, monitoring should be similar to that described for liver transplant recipients but should also include a pre-transplant liver biopsy to establish a baseline.

Summary of recommendations for screening, monitoring and reporting for hepatitis C virus

Liver transplant trials

• Screening
  – Screening for HCV should be performed on all patients awaiting liver transplantation with an EIA ± qualitative PCR.
  – In patients known to be HCV positive, a pre-transplant quantitative HCV viral load should be measured once.
  – Genotyping is recommended in all transplant trials enrolling HCV-infected patients.

• Monitoring
  – HCV RNA quantitative viral loads should be done at regular intervals for HCV-positive patients. A suggested protocol is: baseline, 3, 6, 9 and 12 months post-transplant.
  – Routine surveillance biopsies are recommended. A suggested biopsy protocol is twice within the first year post-transplant: at 4–6 months post-transplant and at 10–12 months post-transplant. Biopsies should also be performed as clinically indicated for elevations of liver enzymes.

• Reporting
  – Reporting should include histologic recurrence as defined by biopsy. The degree of necroinflammatory activity and the degree of fibrosis should be graded on each biopsy using a standard grading system (examples as noted above include Batts and Ludwig system, METAVIR, Knodell Histological Activity Index (HAI)). Severe forms of recurrence as defined above and graft loss due to HCV should be reported.
Nonliver transplant trials

- **Screening**
  - Screening for HCV can be performed on all patients awaiting transplantation with an EIA or a qualitative PCR.
- **Monitoring**
  - Monitoring should be similar to that described above for liver transplant recipients but should also include a pre-transplant liver biopsy.
- **Reporting**
  - Reporting should include HCV disease progression as defined by biopsy. As above, the degree of necroinflammatory activity and the degree of fibrosis should be graded on each biopsy using a standard grading system.

Fungal infections

**Background**

Opportunistic mycoses can cause significant complications in organ transplant recipients. The incidence of invasive fungal infections varies from 2–14% in renal transplant recipients to higher rates, ranging from 7–42% in liver, 15–35% in lung and heart-lung and 40–49% in small bowel recipients to 80% in heart transplant recipients (45–48). The vast majority of these infections are due to Candida (35–91%) or Aspergillus (9–52%) species, with Cryptococcus neoformans, non-Aspergillus mycelial fungi and endemic mycoses accounting for most of the others. Standard definitions will be helpful in interpreting outcomes after immunosuppressive trials (49).

**Clinical definitions**

Proposed definitions for invasive fungal infections for use in clinical trials of immunosuppression are modified from the EORTC/MSG criteria used in patients with cancer and stem cell transplant recipients (50). Only proven and probable infections should be reported in immunosuppression trials.

**Aspergillus and other mold infections**

(i) **Proven:** Histologic or cytopathologic examination showing hyphae from needle aspiration or biopsy specimen with evidence of associated damage (either microscopically or unequivocally by imaging); or positive culture result for a sample obtained by sterile procedure from normally sterile and clinically or radiologically abnormal site consistent with infection, excluding urine and mucous membranes (50). If culture results show very low colony numbers, the possibility of contamination should be excluded. Blood cultures that yield fungi, accompanied by temporally related clinical signs and symptoms compatible with relevant organism are also considered as proven fungal infections (50).

Tracheobronchitis or bronchial anastomotic infection is an entity observed uniquely in lung transplant recipients. Although a locally invasive infection, it has the potential to progress to disseminated infection or result in fatal bronchopleural fistulas. Tracheobronchitis or endobronchial infection is defined as isolation of a fungal pathogen (typically Aspergillus) in culture with histopathologic evidence of tissue invasion or necrosis, ulceration or pseudomembranes on bronchoscopy (51).

(ii) **Probable:** Probable invasive fungal infection due to mycelial fungi is defined as positive culture for mold (including Aspergillus, Fusarium, Scedosporium species, zygomycetes or endemic fungal pathogen) from sputum or bronchoalveolar lavage fluid sample, and any of the following new infiltrates on CT imaging: halo sign, air-crescent sign or cavity within the area of consolidation (50). Patients with symptoms of lower respiratory tract infection with microbiologic evidence of infection and a new infiltrate not fulfilling the above criteria may also be considered as having probable pulmonary infection. In lung transplant recipients, a new infiltrate should be used as a clinical criterion only when bacterial and/or viral infection is ruled out by culture and rejection is excluded by lung biopsy.

Aspergillus can be detected from the respiratory tract of ~1.5% of liver, and ~10% of heart transplant recipients with positive predictive values of 41–72% for liver and 60–70% in heart transplant recipients (46,52). Thus, while a positive culture of Aspergillus in liver and heart transplant recipients may be considered presumptive evidence of infection in clinical practice, in the context of a clinical trial, criteria for probable invasive infection must be fulfilled to define invasive aspergillosis.

In the absence of an Aspergillus culture in respiratory samples, ≥2 positive blood samples yielding a positive galactomannan assay and clinical criteria compatible with probable invasive fungal infection are also considered indicative of probable invasive aspergillosis (50). However, it should be noted that a limited number of studies utilizing the galactomannan test for the diagnosis of invasive aspergillosis in solid organ transplant recipients suggest that it has relatively low sensitivity (30–55.6%) (53–55). In addition, false positive tests may occur in 13–20% of patients (54,55).

**Endemic fungal infections**

(i) **Proven:** A positive culture from the site affected, in a patient with symptoms attributed to fungal infection. If culture results are negative or unattainable, histopathologic or direct microscopic demonstration of appropriate morphological forms is considered adequate for dimorphic fungi (Blastomyces, Coccidioides and Paracoccidioides species) having a truly distinctive appearance (50). It should be noted that Histoplasma capsulatum variant capsulatum may resemble Candida glabrata. In case of histoplasmosis, a diagnosis of disseminated infection may be established by positive blood culture or result of Histoplasma antigen in the urine or serum antigen by means of RIA.
Invasive infections due to yeasts

Invasive candidiasis is defined as isolation of Candida species (i) from the blood or a normally sterile site or (ii) histopathologic and culture evidence of tissue invasion by biopsy or autopsy (49,50). Isolation of Candida spp. in urine, bile, respiratory samples or T-tubes in the absence of above criteria should be considered as colonization (49).

Invasive cryptococcosis is defined as identifying Cryptococcus species in culture of blood, CSF or tissue, and/or by histologic or cytopathologic examination. Antigen positivity for Cryptococcus in blood or cerebrospinal fluid should also be considered as a definite invasive infection (50). False-positive cryptococcal antigen reactions due to infection with Trichosporon beigelii, or Stomatococcus mucilaginosus, circulating rheumatoid factor, or concomitant malignancy should be excluded if positive antigen is the only evidence of infection (50).

Laboratory monitoring

In trials of immunosuppression therapy, surveillance monitoring for fungal infections (e.g. surveillance cultures or antigen-based/molecular-based testing) is not indicated. Microbiologic and radiologic evaluation should be done in response to appropriate clinical symptoms, and ultimate diagnosis should be based on the guidelines described above.

Summary of recommendations for screening, monitoring and reporting for fungal infections

- **Screening**
  - No routine screening

- **Monitoring**
  - No routine monitoring

- **Reporting**
  - The organism, the site of infection, and whether it represents invasive infection or colonization should be reported. Invasive mould infections should be reported as proven or probable as outlined above.

BACTERIAL INFECTIONS

Background

Bacterial infections comprise the most common type of infections reported following organ transplantation. The nature and site of infection are in large part determined by the type of transplant. It is important to describe the frequency and type of bacterial infections occurring in patients enrolled in trials of immunosuppressive therapy.

Definitions

It is proposed that bacterial infections be documented in clinical trials based on a syndromic definition in addition to microbial etiology. The following are common syndromes (i.e. definitions do not encompass all potential bacterial syndromes) occurring after transplantation and proposed definitions for use in clinical trials (56).

Urinary tract infections

1. Asymptomatic bacteriuria: Positive urine culture of >10^8 cfu/L (10^5 cfu/mL) with no symptoms and no evidence of pyuria (≥3/hpf unspun urine or positive leukocyte esterase). As the significance of asymptomatic bacteriuria is unclear, this should not be used as an infectious complication in clinical trials of immunosuppression.

2. Acute uncomplicated cystitis: Defined by a positive urine culture of >10^8 cfu/L (10^5 cfu/mL) in the presence of pyuria. (≥3/hpf unspun urine or positive leukocyte esterase). Patients may have dysuria, frequency and/or urgency, but symptoms are not mandatory for diagnosis since they are often absent, especially in kidney transplant recipients.

3. Acute pyelonephritis or renal allograft pyelonephritis: Pyelonephritis is defined by the presence of a positive urine culture and pyuria in association with tenderness over graft (kidney transplant patients with graft pyelonephritis) or at costovertebral angle (native kidney pyelonephritis) with fever and/or positive blood culture.

4. Perinephric abscess: Defined by presence of a positive culture from a needle or intra-operative drainage of a perinephric collection.

Bacterial pneumonia

Bacterial pneumonia is defined by the presence of one or more of the following symptoms: cough, hemoptysis, fever, dyspnea (or increased oxygen requirement), pleuritic chest pain plus chest X-ray or CT chest findings of new pulmonary parenchymal disease. A definite diagnosis is based upon meeting the above criteria in combination with the presence of a positive sputum, BAL culture or lung biopsy. In the absence of one or more of these criteria, the patient should be considered to have an episode of probable pneumonia.

Empyema: Defined by the presence of a positive culture from pleural fluid obtained in a sterile manner (i.e. thoracentesis or from a newly inserted chest tube)

Intra-abdominal infection

a) Intra-abdominal abscess: The presence of an intra-abdominal abscess can be diagnosed based on results of CT or ultrasound of the abdomen as well as by identification at laparotomy. Microbiologic evidence includes the presence of a positive culture obtained in sterile fashion (i.e. needle biopsy, operative specimen or a new drain). Cultures obtained from existing
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drains may represent colonization; however, if accompanied by new symptoms (fever, abdominal pain) that are compatible with infection and consistent imaging, then it can be considered as probable (recognizing that some cases of colonization may be mistaken as disease).

b) Cholangitis: Fever accompanied with right upper quadrant pain or abnormal liver function tests plus a response to antimicrobial therapy can be classified as probable cholangitis.

Bacteremia

Bacteremia must be documented by a positive blood culture and should be characterized by its source or accompanying syndrome. Examples of potential sources and syndromes include central venous catheter-related bacteremia, pneumonia, intra-abdominal infection, skin/soft tissue infection or meningitis. Contamination should be differentiated from true bacteremia by clinical presentation and if possible by repeated peripheral blood culture rather than from previously placed indwelling catheters. A single positive culture for coagulase negative staphylococcus in the absence of any symptoms is often a contaminant and should not be classified as a definitive bacteremia.

Laboratory monitoring

Generally, no specific monitoring is required for bacterial infections in studies of immunosuppressive therapy. Appropriate cultures should be obtained prior to initiation of antibiotics if a study subject becomes febrile or develops symptoms compatible with a bacterial syndrome.

Summary of recommendations for screening, monitoring and reporting for bacterial infections

- **Screening**
  - No routine screening
- **Monitoring**
  - No routine monitoring
- **Reporting**
  - The infective syndrome (e.g. pneumonia, pyelonephritis) should be reported along with infection. Where applicable, infection should be classified as proven or probable as outlined above.

ARCHIVING OF SPECIMENS

Multi-center trials that have large numbers of subjects with detailed clinical follow-up are a potential valuable source of information. The collection and archiving of specimens as part of a multi-center immunosuppression study serves two functions: (i) it allows for batch testing of samples for the recommended infectious disease laboratory monitoring and (ii) it creates a potential resource for evaluation of new and emerging pathogens and novel diagnostic methodologies. For example, if CMV and BK virus monitoring is to be done monthly, centers can store monthly blood samples and send to a designated central laboratory in batches. Viral load evaluations can then be performed on batches received from several centers at regular intervals throughout the study or at the end of the study. This has several advantages: it facilitates centralized laboratory testing in multi-center trials, saves considerably on costs related to virologic testing and shipping, and simplifies logistical aspects of virologic monitoring in large multi-center trials.

Although to ideally cover the broadest range of pathogens, peripheral blood could be separated into whole blood, plasma and lymphocyte fractions, the labor costs involved in lymphocyte preparation may make this impractical. A more practical recommendation is to collect serial samples of peripheral blood, preferably two tubes at each sampling time, one of which could be aliquoted as whole blood, the other separated and stored as plasma. Rapid specimen transport to the local laboratory is important. Samples should be aliquoted and stored at ~70°C to avoid freezing when multiple assays are being performed. In keeping with the proposals for monitoring outlined in this document, samples can be collected monthly during the first year following transplantation.

Finally, consideration should be given to storage of a pretransplant donor and recipient samples (blood and serum) for evaluation of donor-transmitted infections should the need arise.

CONSENT ISSUES

Inclusion of an infectious disease-monitoring plan in immunosuppressive trials raises two important issues with regard to informed consent. First, we recommend that infectious disease monitoring protocols should be included in the trial design and as such be built into the informed consent process. For example, if CMV will be monitored, a statement such as ‘blood will be collected at monthly intervals for one year following the transplant to test for the presence of a common viral infection after transplant called cytomegalovirus’ should be included in the consent form. Since, for large multi-center trials, the recommended laboratory protocols may not be done in ‘real time’ but rather through batching of specimens to facilitate monitoring, results will generally not be available to guide clinical decisions. Alternatively, a study steering committee may choose to blind all results. This should be made clear in the informed consent. We again emphasize that the proposed monitoring does not supplant clinical monitoring strategies that may already be in place at participating centers. If a steering committee decides that some testing should be available in real-time to guide clinical decision making, then
every effort should be made to define ‘a priori’ cut-points for positive tests, and to standardize clinical responses to positive tests.

The second issue is more complex and involves the use of banked specimens. If a study steering committee decides to bank specimens for possible future research studies, the use of these specimens cannot occur in the absence of appropriate measures to set up a specimen repository and obtaining informed consent from the individual patients. Each study should have an independent steering committee that will determine whether or not the specimens could be used for a secondary study. Federal guidelines outline procedures for setting up such a repository (57,58). These include oversight of an Investigational Review Board (IRB), written, standardized informed consent forms and sample collection protocols. Every attempt should be made to initiate this process prior to implementation of the protocol in order to optimize access to these potentially useful banked specimens.

SUMMARY

As novel immunosuppressive strategies continue to evolve, the type, frequency and presentations of infectious complications associated with transplantation will undoubtedly continue to change. We propose these definitions and guidelines for laboratory monitoring as the first step to standardization of infectious disease monitoring in immunosuppressive trials. Inclusion of definitions and documentation of infectious disease complications will provide critical information regarding the clinical impact of new immunosuppressive regimens. It is expected that these recommendations will continue to evolve in response to the changing epidemiology of infectious disease after transplant and due to the development of new diagnostic modalities. Ultimately, it is our hope that the widespread adoption and continuing evolution of these or similar recommendations lead to a better understanding and to new discoveries about infectious risks related to transplantation.

References