

## Special Article

# Herpes Simplex Virus in Solid Organ Transplantation

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**Abbreviations:** BAL, bronchoalveolar lavage; CMV, cytomegalovirus; CSF, cerebrospinal fluid; DFA, direct fluorescent antibody; EBV, Epstein-Barr virus; GFR, glomerular filtration rate; HSV, herpes simplex virus; IgG, immunoglobulin G; IgM, immunoglobulin M; PCR, polymerase chain reaction; SOT, solid organ transplant.

## Herpes Simplex Virus (HSV) 1 and 2

### Epidemiology

Herpes simplex virus type-1 and 2 (HSV-1, HSV-2) are  $\alpha$ -herpesviruses which establish latency in nerve root ganglia. Infection with HSV-1, the classic oro-labial herpes virus, is acquired from early childhood through adulthood, with prevalence in the United States of 44% in 12–19 year olds and approximately 80% by the age of 60 (1). Associated primarily with genital herpes, HSV-2 has a seroprevalence that increases rapidly at the age of sexual debut, infecting 1.6% of persons aged 14–19 years and 26.3% of persons aged 40–49 years in the United States (2). In recent years, HSV-1 is an increasing cause of genital lesions, though typically with less frequent recurrences (3,4). Most adult transplant patients are infected with HSV-1 or HSV-2, or both, with prevalence similar to the distribution by age in the general population.

A minority of immunocompetent persons infected with HSV develop symptomatic lesions; however, most will shed virus on mucosal surfaces (5). Compared with immunocompetent persons, solid organ transplant (SOT) recipients shed virus more frequently, have more frequent and severe clinical manifestations of HSV (6,7), and may be slower to respond to therapy. Most symptomatic HSV disease in adult transplant recipients results from reactivation

of previously acquired virus, particularly early after transplantation and in the setting of antirejection therapy (8–10). Primary infection from the allograft is rare but described in liver, kidney and other organ transplant types (10–13). Patients may present early after transplant with a fulminant course with hepatitis and poor outcome. HSV seronegative SOT recipients may also acquire HSV from intimate contacts.

The most common clinical presentation of HSV is orolabial, genital or perianal disease (8,9). Lesions can be classic vesicular and/or ulcerative and may extend locally. Visceral or disseminated disease can occur, including disseminated mucocutaneous disease, esophagitis, hepatitis and pneumonitis (14,15). Fever, leucopenia and hepatitis are the most common presenting signs of disseminated disease. Pneumonitis is described in recipients of all organ types, but is most common in heart–lung transplant recipients (15). Rarely, visceral disease may occur in the absence of cutaneous or mucosal findings.

Keratitis (infection of the cornea) is the most common manifestation of HSV in the eye (16). Keratitis presents in a variety of pathophysiologic entities. Superficial infection has historically been thought to result from HSV infection in the trigeminal nerve. However, other, pathology may be the result of deeper infection of corneal tissues (e.g. stromal keratitis) with resultant inflammatory reaction and/or immune mediated responses to remaining antigen (17).

### Risk factors

Recipient HSV IgG serostatus should be determined prior to transplant (II-1). It should be noted that there is limited utility in testing infants and children in the first 6–12 months of life when they may still harbor maternal antibodies. HSV seropositive recipients are at risk of clinical reactivation posttransplant in the absence of antiviral prophylaxis even if they had not had prior clinical HSV disease. The incidence of clinically apparent HSV disease in HSV-seropositive adult transplant patients who are not receiving antiviral prophylaxis ranges from 35% to 68% (9,10,18). Because severe HSV disease can occur in HSV-seropositive or in seronegative persons who newly acquire the infection, HSV infection should be considered in the differential diagnosis of clinically appropriate syndromes regardless of serostatus prior to transplantation. Knowledge of serostatus may be important to determine the possibility of primary HSV acquisition, either from the allograft or from natural sources after transplant, which may be more clinically severe and prolonged due to lack of immunologic memory (19,20).

**Table 1:** Laboratory methods for diagnosis of HSV

Test	Advantage	Disadvantage
Direct fluorescent antibody (DFA)	<ul style="list-style-type: none"> <li>● Rapidly available</li> <li>● Virus-specific</li> </ul>	<ul style="list-style-type: none"> <li>● Lower sensitivity than PCR</li> <li>● Limited sample types (needs cells to stain; e.g. not CSF)</li> </ul>
PCR	<ul style="list-style-type: none"> <li>● Most sensitive</li> <li>● Done on most sample types</li> </ul>	<ul style="list-style-type: none"> <li>● Not available at all centers</li> <li>● Positive result, other than in CSF, requires interpretation</li> </ul>
Culture	<ul style="list-style-type: none"> <li>● Type-specific</li> <li>● Able to isolate virus for drug susceptibility testing</li> </ul>	<ul style="list-style-type: none"> <li>● Takes longer</li> <li>● Less sensitive, only ~25% of PCR positive depends on level of virus (Ref. 23)</li> </ul>
Tzank smear	<ul style="list-style-type: none"> <li>● Rapid</li> <li>● Direct visualization</li> </ul>	<ul style="list-style-type: none"> <li>● Requires experience</li> <li>● Nonspecific (HSV or VZV)</li> </ul>
Histopathology with immunohistochemistry	<ul style="list-style-type: none"> <li>● Can prove tissue-invasive disease</li> </ul>	<ul style="list-style-type: none"> <li>● Samples more difficult to acquire</li> <li>● Long turnaround</li> </ul>
Serology	<ul style="list-style-type: none"> <li>● Useful to guide pretransplant risk stratification and prevention</li> </ul>	<ul style="list-style-type: none"> <li>● Not useful posttransplant, insensitive marker for acute infection</li> <li>● False-positive IgM with HSV reactivation</li> </ul>

The incidence of HSV reactivation with specific immunosuppressive regimens has not been formally assessed. Historically, use of anti-CD3 antibody muromonab (OKT3) and mycophenolate mofetil have been associated with an increased risk of HSV reactivation after transplantation (10,21,22). There have been no evaluations to date comparing different induction regimens (T cell depleting agents such as rabbit-antithymocyte globulin or alemtuzumab vs. nondepleting agents such as basiliximab or daclizumab) or maintenance immunosuppressive regimens with regards to HSV reactivation rates. However, there are some data to suggest use of the mTOR inhibitors (e.g. rapamycin) with reduced calcineurin inhibitor exposure leads to reduced herpes virus infections (23,24).

**Diagnosis (Table 1)**

Although most patients present with typical orolabial and genital lesions, HSV in immunocompromised hosts may be atypical, thus, laboratory confirmation may be helpful. HSV grows well in tissue culture so that most isolates are identified within 5 days. Timing of sampling is important with mucocutaneous lesions: for example, sampling of genital lesions >5 days old had a yield of less than 35% (25). Direct fluorescent antibody (DFA) testing of mucocutaneous lesions, bronchoalveolar lavage (BAL) and other clinical samples, can provide rapid results. Compared with virus isolation the sensitivity has been reported between 60% and 75% and specificity of 85–99% (25–27). Polymerase chain reaction (PCR) assays are up to fourfold more sensitive than tissue culture for diagnosing mucocutaneous HSV and have replaced viral culture as the preferred diagnostic test (28–32), culture and DFA remain options for mucocutaneous lesions. The use of PCR in cerebrospinal fluid to diagnose HSV encephalitis is the diagnostic test of choice, with a sensitivity of 98% and specificity approaching 100% (33). HSV DNA is also detected in the blood of immunocompetent patients with primary ulcerative infection (34) and in those with significant reactivation disease (34,35); however, the clinical significance of finding HSV DNA in the blood outside of patients with clinical syndromes consistent with disseminated disease has not been well established (36). Tissue histopathology with im-

munocytochemistry for HSV, can be helpful and is recommended to confirm a diagnosis where PCR or other tests (e.g. culture) may represent contamination from another site (e.g. BAL contaminated from oropharynx). Serologic testing is rarely useful for diagnosing acute infections as most patients will be HSV seropositive and IgM positivity in HSV infection may indicate reactivation and not new acquisition. Nevertheless, serology (by IgG) is useful to acquire pretransplant for appropriate posttransplant risk stratification.

Diagnosis of HSV keratitis remains primarily a clinical diagnosis based on characteristic features of the corneal lesion on slit lamp microscopy. Referral to an ophthalmologist is requisite for appropriate diagnosis and treatment of HSV ocular disease (Table 1).

**Prevention**

Currently, many transplant recipients receive antiviral medication to prevent CMV replication (see CMV guidelines). Ganciclovir (Grade I for HSV prevention), acyclovir (I), valacyclovir (I) and valganciclovir (III), prevent most HSV replication when given in standard doses for CMV prevention. HSV-specific prophylaxis should be considered for all HSV-1 and HSV-2 seropositive organ recipients not receiving antiviral medication for CMV prevention (Grade I). Some centers use EBV-specific prophylaxis in pediatric transplant recipients not receiving prophylaxis for CMV infection. The antivirals used for EBV prevention also likely prevent HSV reactivation, so additional prophylaxis may not be necessary (Grade III). In the unusual circumstance of a patient who is not receiving CMV antiviral prophylaxis and is also HSV seronegative, the risk of early posttransplant HSV infection is not well defined, though probable cases of HSV transmission from organs have been described (11). In this setting, some clinicians may choose to give antiviral prophylaxis while others may consider close clinical monitoring (Grade III).

Immunosuppression intensification for organ rejection has been associated with HSV recurrence, though usually not life threatening. Limited data suggest that prophylaxis during rejection episodes treated with OKT3 is effective (21);

and the utility of HSV prophylaxis is likely similar for other types of immunosuppressive regimens (Grade II-2). Patients may also be receiving antivirals for CMV prophylaxis during treatment of rejection so HSV-specific prophylaxis may not be required.

Unfortunately, a vaccine to prevent primary HSV infection has been elusive, therefore current prevention techniques are focused on behavioral and antiviral methods to prevent acquisition of HSV. Seronegative transplant recipients should be counseled regarding the risks of HSV-1 and HSV-2 acquisition. It is important to avoid contact with persons with active lesions as these patients are most infectious (Grade III). However, persons may acquire HSV from asymptomatic individuals so care should be taken in intimate contact, particularly during periods of most intense immune suppression (Grade III). Condoms may be effective, but do not completely protect against HSV transmission (37). A majority of persons infected with HSV have never had symptomatic lesions, so the virus may be acquired from persons who have never had lesions. Where appropriate, HSV-2 seronegative transplant recipients in new sexual relationships should consider having their partner tested for HSV-2 (Grade III). In serodiscordant couples, daily antiviral therapy taken by the seropositive partner has been shown to prevent HSV-2 transmission to the seronegative partner (38), so this may be considered as an option (Grade III), but has not been evaluated in the SOT population. There are no controlled studies looking at the efficacy of postexposure prophylaxis to prevent HSV acquisition so it is not routinely recommended.

**Antiviral dosing for prophylaxis (Table 2):** The only randomized trials of HSV prophylaxis in SOT recipients were published in the 1980s and showed effective HSV suppression with acyclovir administered at doses of 200 mg three (9) or four (8) times a day. In a meta-analysis comparing these regimens with higher doses of acyclovir and valacyclovir for CMV prevention, HSV was well suppressed at all evaluated doses of acyclovir, with no difference between these “low-dose” (<1 g/day) and the higher dose regimens (39); Table 2). In this meta-analysis, the use of acyclovir resulted in a significant reduction (OR, 0.17; 95% CI, 0.12–0.24;  $p < 0.001$ ) in HSV disease (39).

Compared with these initial HSV prevention trials in SOT, higher doses of acyclovir administered less frequently (e.g. 400–800 mg 2×/day) have been shown to be safe and effective in other similarly immunocompromised populations (e.g. hematopoietic stem cell transplant, HIV), and are recommended for SOT recipients due to their safety and ease of administration (Grade II). Because SOT-specific studies have not been done, the level of evidence reported herein is extrapolated from studies performed in populations of other patients with similar levels of immune compromise (40–42). Patients with a history of frequent severe clinical HSV reactivations prior to transplant should be given doses in the higher range (Grade III). Valacyclovir given twice daily was found to be superior to once daily when

used as prophylaxis against HSV in immunocompromised patients so once daily administration is generally not recommended (43). Dosage adjustment for renal insufficiency is necessary if GFR is <50.

Famciclovir, the oral prodrug of penciclovir, is also effective in preventing recurrent HSV in immunocompromised and immunocompetent hosts (44,45) and is an alternate option for prophylaxis.

HSV prophylaxis in pediatric patients is not universal. Dosing for seropositive patients or patients who have had prior occurrences is derived from studies of HIV positive and stem cell transplant recipients. For children  $\geq 2$  years of age requiring oral therapy a typical quantity is 600–1000 mg/day in 3–5 divided doses. For intravenous therapy, 5 mg/kg every 8 h is recommended (46).

**Duration of prophylaxis:** The majority of severe HSV disease occurs within the first month after transplant (9), so antiviral prophylaxis should continue for at least a month (Grade I). In addition, resumption of prophylaxis may be considered for patients being treated for rejection (with T cell depleting agents) (Grade III). For patients receiving CMV antiviral prophylaxis (typically continued for  $\geq 100$  days), additional HSV prevention is not necessary. In patients who experience bothersome clinical recurrences ( $\geq 2$ ) after discontinuation of antiviral therapy, suppressive antiviral therapy should be continued until such time as the level of immunosuppression can be decreased (Grade I). Of note, suppressive therapy can be safely continued for many years and is associated with less frequent acyclovir resistant HSV than episodic therapy in immunocompromised patients (41), and thus is the preferred approach (Grade III). If cessation of prophylaxis is unsuccessful, then lifelong suppressive therapy may be necessary (Grade III).

#### **Treatment (Table 2)**

Disseminated, visceral, or extensive cutaneous or mucosal HSV disease should be treated with intravenous acyclovir (Grade II-1) at a dose of 5–10 mg/kg every 8 h (11,14,42,47,48). Mucocutaneous disease in the immunocompromised patient can be treated with the lower dose of 5 mg/kg. When there is a concern for disseminated, visceral or cerebral involvement doses of up to 10 mg/kg every 8 h should be initiated (with adjustment for reduced GFR) (Grade II). Rapid initiation of acyclovir therapy is associated with improved outcome for HSV disease in transplantation (11), and can be life-saving in cases of HSV hepatitis or dissemination. Reduction in immunosuppression should be considered for life-threatening HSV disease (Grade III). More limited mucocutaneous disease can be treated with oral acyclovir (I), valacyclovir (I) or famciclovir (I). Therapy should be continued for minimum of 5–7 days or until complete healing of the lesions depending on the clinical circumstances. Therapy in severe disease (e.g. encephalitis) should be continued for a minimum of 14 days (Grade III) although some clinicians favor longer courses up to 21 days (49–51).

**Table 2:** Recommendations for HSV prevention and treatment in HSV seropositive solid organ transplant recipients

Indication	Treatment	Evidence	Comments
<b>Prevention</b>			
Adult:	CMV prophylaxis <sup>1</sup> or ACV 400–800 mg p.o. 2×/day VACV 500 mg p.o. bid FCV 500 mg p.o. bid	Grade I Grade I Grade I Grade I	<ul style="list-style-type: none"> <li>• Administer for at least 1 month</li> <li>• During treatment of rejection episodes (for at least 1 month)</li> </ul>
Pediatric:	ACV 30–80 mg/kg p.o. in 3 divided doses VACV 15–30 mg/kg/p.o. tid	Grade III	<ul style="list-style-type: none"> <li>• For recurrent infection: Lower doses for recurrent labialis, higher doses for recurrent genital or ocular disease.</li> </ul>
<b>Treatment</b>			
<b>Mucocutaneous disease</b>			
Adult:	ACV 400 mg p.o. 3×/day VACV 1 g p.o. 2×/day FCV 500 mg p.o. 2×/day ACV 5 mg/kg i.v. every 8 h (if unable to take p.o.)	Grade I Grade I Grade I	<ul style="list-style-type: none"> <li>• Because prompt initiation of therapy is associated with improved outcome, therapy should be started based on clinical diagnosis, pending laboratory confirmation</li> <li>• Therapy should be continued until complete healing of all lesions or at least 5–7 days</li> <li>• Severe mucocutaneous.</li> <li>• Limited disease. Treat for 7–14 days.</li> </ul>
Pediatric:	ACV 10 mg/kg i.v. every 8 h ACV 1000 mg/day p.o. in 3–5 divided doses for 7–14 days		
<b>Severe, visceral/disseminated/CNS disease</b>			
Adult:	ACV 10 mg/kg i.v. every 8 h	Grade II-1	<ul style="list-style-type: none"> <li>• i.v. Therapy should be continued until resolution of disease, or 14 days, then oral medication may be given. For CNS infection may consider 21 days of IV therapy.</li> </ul>
Pediatric:	ACV IV 60 mg/kg/day in 3 divided doses	Grade II-2	<ul style="list-style-type: none"> <li>• Continue for 21 days for disseminated or CNS infection.</li> </ul>
<b>HSV Keratitis</b>			
	Topical: Ganciclovir 0.15% Trifluorothymidine 1% Acyclovir 3% ointment (Grade III) Acyclovir, 400 mg five times daily Valacyclovir and Famciclovir	Grade I  Grade I Grade III	<ul style="list-style-type: none"> <li>• Topical steroids should also be considered for stromal keratitis.</li> <li>• Ganciclovir given 5 × a day until healing then 3 × daily for 1 week</li> <li>• One drop every 2 h for 2 weeks. Limited by epithelial toxicity</li> <li>Avoids topical toxicity</li> <li>No comparative or dose finding studies.</li> </ul>
	Oral: Foscarnet 80–120 mg/kg/day IV in 2–3 divided doses until healing is complete	Grade I	<ul style="list-style-type: none"> <li>• Resistance should be laboratory-confirmed, although empiric therapy can be started</li> </ul>
<b>Acyclovir-resistant HSV</b>			
	Intravenous cidofovir	Grade II-3	<ul style="list-style-type: none"> <li>• Reduce immunosuppression, if possible</li> </ul>
	Topical cidofovir	Grade III	
	Topical trifluridine	Grade II-3	

ACV = acyclovir; CMV = cytomegalovirus; FCV = famciclovir; HSV = herpes simplex virus; i.v. = intravenously; p.o. = per orally; SOT = solid organ transplant; VACV = valacyclovir.

<sup>1</sup>CMV prophylaxis with recommended doses of ganciclovir, valganciclovir, valacyclovir or acyclovir are adequate for HSV prevention.

Due to lack of SOT-specific studies, the level of evidence is extrapolated from populations of other patients with similar levels of immune compromise. Dosages are for GFR ≥ 50, adjustment is necessary for renal insufficiency.

Children clear acyclovir more rapidly than adults, and thus need higher doses of acyclovir. There are no controlled clinical trial data for dosing of anti-HSV medications in the SOT pediatric population. In neonates, the recommended dose of acyclovir for encephalitis is 20 mg/kg/dose every 8 h for 21 days (52). Persistent HSV PCR in CSF has been associated with poor outcome in neonatal infection and it is suggested to confirm a negative CSF PCR prior to completing therapy (53) (Grade III). A similar dose is recommended for children from 3 months to 12 years, although some clinicians prefer 15 mg/kg/dose every 8 h (46). Local-

ized, mucocutaneous, progressive disease is treated with IV acyclovir at a dose of 10 mg/kg/dose every 8 h for a minimum of 14 days (Grade III). For less severe localized disease oral acyclovir may be used at a dose of 1000 mg/day in 3–5 divided doses for 7–14 days; maximum dose: 80 mg/kg/day not to exceed 1 g/day (46). Acyclovir is associated with greater toxicity in the pediatric population; thus, close monitoring is recommended. Data for oral valacyclovir come from healthy immunocompetent patients: a dose of 20 mg/kg/dose twice daily is recommended for children 3 months to 11 years of age (54). Valacyclovir is

FDA approved for treatment of herpes labialis in children over 12 years of age and for children  $\geq 2$  years of age for the treatment of varicella infection though is not always easily available to the pediatric population as it needs to be reconstituted soon before use to be in liquid form.

HSV keratitis treatment includes both topical and/or systemic therapy. The various forms of topical therapy appear equally effective (55). Topical agents such as trifluridine solution and vidarabine ointment may result in epithelial toxicity with prolonged use. Topical ganciclovir gel has also been shown to be effective and has the advantage of less toxicity and less frequent applications. A study in immunocompetent individuals showed acyclovir at a dose of 400 mg five times a day was equivalent to topical therapy (56) and avoids the epithelial toxicity. Alternate HSV medications such as valacyclovir or famciclovir are possibly as effective as acyclovir, but have not been studied in comparative trials (57). Stromal keratitis and endotheliitis is treated with a combination of antivirals and topical steroids (58).

### Resistance

The estimated prevalence of acyclovir resistance in immunocompromised hosts ranges from 3.6% to 6.3% (59,60) and needs to be considered in patients whose lesions are not responding clinically to appropriate doses of acyclovir, valacyclovir or famciclovir therapy. The most common mechanism of resistance in clinical practice is due to diminished or absent thymidine kinase (TK) activity that is conferred by resistance mutations. Thus, drugs that utilize TK (acyclovir, famciclovir and valacyclovir) are all affected. Initial evaluation should include laboratory confirmation of HSV disease including a viral culture as testing for acyclovir resistance generally relies on phenotypic assays—most commonly a plaque reduction assay. Given that testing relies on growth of the virus, results may be delayed for days to weeks and when strongly suspected, alternate therapy should be considered prior to confirmation of resistance (Grade III). Genotypic testing for known resistance mutations is available in some settings and may have a more rapid turnaround time.

Foscarnet is recommended for acyclovir resistant HSV infections (Grade I) (61). Intravenous cidofovir (Grade II-3) has also been associated with improvement (62), but both of these drugs are associated with significant renal toxicity and appropriate care should be taken to monitor for toxicities of these alternative regimens. Probenecid is usually given with cidofovir to potentiate the toxicity. Topical imiquimod has also been used for resistant anogenital HSV in immunocompromised hosts (63,64). Topical cidofovir (Grade III) and trifluridine (Grade II-3) have also been used. Oral lipid-ester formulations of cidofovir (CMX-001) and helicase-primase inhibitors (e.g. ASP2151) are currently in later stages of development and may be available in the near future (65,66). To the extent possible, doses of immunosuppressive therapy should be reduced in patients with acyclovir resistant disease (Grade III). Recurrent acyclovir-resistant HSV disease may

require repeated courses of foscarnet. However, after complete healing, subsequent recurrences may be again susceptible to acyclovir therapy (67).

### Research Issues

The utility of molecular diagnostic testing in tissue and fluids other than CSF (i.e. blood, ascites, BAL) for diagnostic and monitoring purposes requires additional research to establish its role in routine care. Research into the epidemiology and natural history of HSV, in addition to controlled treatment trials are sorely needed in the pediatric population. It is important to further elucidate the effect of different immunosuppressive regimens on the natural history of herpes simplex reactivation and disease, and the potential benefit of suppressive therapy during long-term immunosuppression. As new therapeutic agents become available for HSV, they should be evaluated in the setting of transplant and other immunocompromised hosts. Should a therapeutic or prophylactic vaccine become available, the efficacy and, in the setting of a live virus vaccine, safety in the transplant population will need to be evaluated. The optimal method and duration for HSV prevention in seronegative recipients who are not taking CMV antiviral prophylaxis should be investigated.

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