Herpesviruses are a ubiquitous family of DNA viruses, with 40–60% of the adult population seropositive for cytomegalovirus (CMV) \(^1\), and more than 90% for Epstein-Barr virus (EBV) \(^2\) and varicella zoster virus (VZV). Following primary infection, herpesviruses become latent in the host and may reactivate during periods of immunosuppression, such as in transplant recipients, HIV-positive patients and pregnant women. The clinical course following reactivation presents a screening and diagnostic challenge \(^3\), particularly as reactivation in immunosuppressed patients may have a clinical presentation consistent with many different infections. For example, CMV pneumonitis produces disease clinically consistent with other viral pneumonias such as influenza infection. It is, therefore, imperative to obtain as relevant and accurate diagnostic information to correctly diagnose herpesvirus infections.

**Methods available for diagnosis of herpesviruses**

Both the prevalence and consequences of herpesvirus infection show constant evolution. Reflecting worldwide trends, in Australia herpes simplex virus-1 (HSV-1) is now the dominant strain isolated from genital herpes in patients under 20 years of age, despite the previous more typical association of HSV-1 with orolabial infections \(^4\). Similarly, the clinical significance of herpesvirus infection is changing with increasing numbers of more highly immunocompromised patients, necessitating improvement in diagnosis and management \(^5,6\).

Virological and serological methods for diagnosing herpesvirus infections are widely available and diagnostic techniques are similar for different herpesviruses – serology, nucleic acid test (NAT) assays – although diagnostic approaches vary.

Diagnostic methods are constantly improving, with a trend towards the use of several test types, such as more direct viral detection techniques, including NAT (Table 1). Such assays are of particular use in clinical situations where serological diagnosis is limited, including in highly immunosuppressed patients who do not produce antibody in the same way as immunocompetent hosts \(^5\), and in pregnancy, where maternal immune suppression combined with technically difficult sampling and persistent detection of IgM with sensitive enzyme immunoassays (EIA), hinders accurate diagnosis.

**Differential diagnosis of common syndromes associated with herpesvirus infections**

The spectrum of disease associated with herpesvirus infection is broad, as a single type of herpesvirus may infect multiple cell types \(^7\), and the illness caused by different herpesviruses may be similar (Table 2). Infection in immunocompetent individuals with herpesviruses may often be asymptomatic \(^8\). The rates of symptomatic illness vary between different age groups, with children often presenting less severe illness from betaherpesviruses such as CMV, or gammaherpesviruses such as EBV, but more severe illness from alphaherpesviruses such as HSV.

**Particular diagnostic difficulties**

**Pregnancy**

Maternal or foetal infection with some herpesviruses (particularly CMV and, to a lesser extent, HSV and VZV) is associated with a range of adverse outcomes of pregnancy \(^9,10\). These include stillbirth \(^11\), low birthweight, preterm delivery \(^12\), intrauterine growth restriction \(^13,14\), congenital malformations, developmental delay and long-term neurological sequelae such as sensorineural hearing loss \(^15,16\). Primary maternal infection was thought to pose a greater risk of adverse outcomes than reactivation
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during pregnancy, although more recently studies of CMV have demonstrated higher rates of transplacental transmission during primary infection, but similar disease outcomes in the infant from both primary maternal CMV and maternal CMV reactivation. Diagnostically, it remains important to distinguish between maternal primary and reactivation infection 1.

Risk factor-based selective screening is often unreliable 16, which suggests that antenatal screening of a wider population of pregnant women is of greater benefit in pregnancy management. One possible advantage of screening is early detection of congenital infection, which may provide an opportunity for intervention, such as antiviral or immunoglobulin therapy for the foetus in order to mitigate the clinical disease and improve outcomes. Currently, there are varying recommendations for antenatal screening for different herpesviruses, and routine screening for the more common infections such as CMV is not currently performed in Australia. Serological screening for pregnant women has been identified as an important issue for women at risk, particularly in the context of the asymptomatic nature of many infections, such as CMV and HSV, and high seroprevalence (40–60% for CMV, 20–30% for HSV-2 and ~60% for HSV-1) 15,17. Antenatal serological screening of women for VZV IgG is recommended in Australia 16,18-20, but is not routine 18. Routine serological screening for CMV infection in pregnant women is not currently recommended 16,23, despite it being the most common cause of congenital infection in Australia. However, screening is undertaken in women at risk of CMV infection, such as healthcare and childcare workers and future screening of all pregnant women is being considered.

Congenital infection

There is pathogenic potential for all eight human herpesviruses in newborn infants. Vertical transmission of herpesviruses may occur transplacentally, through ascending infection, during labour and delivery or through contact in the neonatal period 15. CMV is the most common viral cause of congenital infection 21-27 occurring in 0.2–2% of all pregnancies in developed countries 22,25,27,28. Congenital CMV infection is associated with long-term neurological sequelae and deafness 16. Maternal infection with HSV during pregnancy may result in preterm delivery, IUGR or congenital and neonatal disease 29. Maternal infection with VZV in pregnancy is most commonly uncomplicated but may rarely cause foetal death or congenital varicella syndrome 30. Transplacental transmission of EBV, human herpesvirus 6 (HHV-6), human herpesvirus 7 (HHV-7) and human herpesvirus 8 (HHV-8) are rare events and not yet well understood 31-34.

Table 1: Methods available for the diagnosis of herpesvirus infections.

<table>
<thead>
<tr>
<th>Test type</th>
<th>Available methods</th>
<th>Typical use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serology</td>
<td>Enzyme immunoassay</td>
<td>HSV-1</td>
</tr>
<tr>
<td></td>
<td>Immunoglobulin G</td>
<td>HSV-2</td>
</tr>
<tr>
<td></td>
<td>Immunoglobulin M</td>
<td>VZV</td>
</tr>
<tr>
<td></td>
<td>Immunoglobulin A</td>
<td>CMV</td>
</tr>
<tr>
<td></td>
<td>Complement fixation test</td>
<td>EBV (more complex)</td>
</tr>
<tr>
<td></td>
<td>Latex agglutination</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Immunofluorescence</td>
<td></td>
</tr>
<tr>
<td>Nucleic acid test (NAT)</td>
<td>Polymerase chain reaction (PCR)</td>
<td>All</td>
</tr>
<tr>
<td></td>
<td>Quantitative PCR</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Branch DNA (bDNA)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nucleic acid sequence-based amplification</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(NASBA)</td>
<td></td>
</tr>
<tr>
<td>Antigen detection</td>
<td>Direct fluorescent antibody</td>
<td>HSV-1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HSV-2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VZV</td>
</tr>
<tr>
<td>Culture</td>
<td>Vero cells</td>
<td>HSV-1</td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td>HSV-2</td>
</tr>
<tr>
<td></td>
<td>Fibroblasts (e.g. MRC-5)</td>
<td>HSV-1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HSV-2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CMV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VZV</td>
</tr>
</tbody>
</table>

The diagnosis of herpesvirus infection during pregnancy may be an outcome of routine screening, diagnosis of maternal infection or diagnosis of foetal infection. Foetuses at risk of infection may be assessed through non-invasive and invasive methods. Ultrasound is a non-invasive investigation used to detect foetal abnormalities 13. However, unremarkable sonographic findings do not exclude the possibility of foetal infection 35 as sensitivity is low 36,96. Invasive methods of prenatal diagnosis are commonly
based on viral detection in samples that are representative of the foetus \(^3\). Appropriate samples include amniotic fluid, cord blood and chorionic villi obtained through amniocentesis, cordocentesis and chorionic villus sampling, respectively \(^14\). Specimens may be tested by PCR and viral culture \(^36\) and viral load can be determined using real-time PCR, which has been shown to be related to disease in adults, although the role of viral load in congenital infections is less well-defined \(^23\)\(^,\)\(^37\)\(^,\)\(^38\). Invasive diagnosis of foetal infection is associated with risk to the foetus which must be balanced with the benefits of diagnosis, and consequently diagnosis of congenital infection is often not made until after birth \(^36\).

**Intracerebral disease**

Intracerebral herpesvirus infections require diagnosis by means that are rapid, sensitive and minimally invasive. Serum and cerebrospinal fluid (CSF) antibodies are not suitable for rapid diagnosis, as titres rarely increase prior to 10 days from the onset of illness \(^39\). Brain biopsy has formerly been the reference standard for the diagnosis of intracerebral herpesvirus infections, but CSF PCR provides rapid diagnosis using small samples obtained through minimally invasive methods with sensitivity and specificity comparable to brain biopsy \(^40\). Despite significant amplification of viral DNA by CSF PCR, in cases such as HSV encephalitis, sensitivity may be limited by low viral load in the CSF \(^41\).

**Immunocompromised patients**

The reactivation of latent herpesvirus infections, notably CMV, in immunocompromised hosts is associated with significant morbidity and mortality \(^3\). The clinical presentation of reactivation is highly variable. Features may be localised or systemic and markedly different from those of the primary infection \(^43\). Furthermore, the risk of reactivation and nature of presentation are influenced by the type of immune compromise. For example, CMV retinitis accounts for >85% of severe CMV disease seen in patients with acquired immunodeficiency syndrome, but less than 5% in solid and bone marrow transplant patients \(^43\). This necessitates the use of methods for the monitoring and rapid diagnosis of such reactivations \(^5\).

In addition, the increasingly widespread use of prophylactic antiviral therapy, particularly in transplant recipients, has contributed to the emergence of antiviral-resistant CMV strains \(^44\). Therefore, the diagnostic tests utilised by clinicians for patient management are constantly evolving. There is growing need to incorporate antiviral susceptibility testing into diagnostic protocols to optimise patient care, particularly of immunocompromised patients with increasing viral load or progression of viral disease.

In immunocompromised patients, the production of antibody in response to viral infection may be attenuated or absent. Consequently, the diagnostic applications of serology in such situations are more limited \(^45\). The use of direct detection of viral antigens and NAT assays allows for the diagnosis of infection in immunocompromised hosts earlier in the course of infection and in the absence of a measurable serological response \(^1\)\(^,\)\(^45\). Fibroblast cell culture is appropriate but time-consuming \(^45\). Thus, direct detection with PCR and antigenaemia assays are the methods of choice in the diagnosis of herpesvirus infection in such settings. They provide rapid diagnosis and the shortest window period between the onset of symptoms and the possibility of a positive test \(^45\). This allows for the optimisation of clinical management, including administration of antiviral medication \(^45\).

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**Table 2: Differential diagnoses of some common syndromes associated with herpesvirus infections.**

<table>
<thead>
<tr>
<th>Infectious mononucleosis</th>
<th>Oral and genital ulceration</th>
<th>Congenital infection with protean clinical manifestations*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Infectious causes:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EBV (80%)</td>
<td>Infectious causes:</td>
<td>Toxoplasma gondii</td>
</tr>
<tr>
<td>CMV (5-10%)</td>
<td>HSV-1</td>
<td>Rubella</td>
</tr>
<tr>
<td><em>Toxoplasma gondii</em></td>
<td>HSV-2</td>
<td>CMV</td>
</tr>
<tr>
<td>Primary HIV</td>
<td>Treponema pallidum</td>
<td>HSV-1</td>
</tr>
<tr>
<td>HHV-6</td>
<td>Haemophilus ducreyi</td>
<td>HSV-2</td>
</tr>
<tr>
<td>HAV (Hepatitis A virus)</td>
<td>Klebsiella granulomatis</td>
<td>Treponema pallidum</td>
</tr>
<tr>
<td>HBV (Hepatitis B virus)</td>
<td>HIV-specific ulceration</td>
<td>VZV</td>
</tr>
<tr>
<td>Rubella</td>
<td>Non-infectious causes:</td>
<td>HIV</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>Connective tissue disease</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hypersensitivity reaction</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lymphoma</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leukaemia</td>
<td></td>
</tr>
</tbody>
</table>

**Infectious causes:**

HSV-1
HSV-2
Treponema pallidum
Haemophilus ducreyi
Klebsiella granulomatis
HIV-specific ulceration
Non-infectious causes:
Drug eruption
Trauma
Manifestation of systemic illness
Neoplasm

* HBV and Parvovirus B19 can also cause disseminated disease but generally present with a syndrome that is clinically distinct.
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Conclusions

Herpesviruses are ubiquitous with continuously evolving epidemiology.

A range of methods is available for the diagnosis of herpesviruses.

Herpesviruses infection may be asymptomatic or associated with a broad spectrum of clinical features.

Herpesvirus infection poses specific diagnostic challenges particularly in the context of immunosuppressed, intracerebral infection, pregnancy and congenital infection.

References

10. ibid., 89–94.