Diagnostic aspects of human alphaherpesvirus infections in dermato-venerology

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1 General introduction

1.1 Herpesviruses, important to mankind

The herpesviruses (Herpesviridae) belong to a family of double-stranded DNA viruses. The origin and the evolution of herpesviruses is not known but presumably began about 200 Myears before present at the same time as the differentiation of species arose. An argument in support of this hypothesis is that more than 100 different herpesviruses have been characterized. The host range is confined to eukaryotes and extends from fungi to the invertebrates and the vertebrates. For example herpesviruses have been found in insects, shellfishes, fishes, reptiles, birds and mammals, including primates. The family of herpesviruses is subdivided in three subfamilies. Alphaherpesvirinae are rapidly growing cytolytic viruses that have the capacity to establish latent infections primarily but not exclusively in nerve ganglia; they have a variable host range. Betaherpesvirinae, which have a restricted host range, are slowly growing “cytomegalic” viruses. Infected cells often become enlarged (“cytomegalia”) and cell lysis occurs several days after infection. Secretory glands, lymphoreticular tissue, kidneys and other organ tissue can harbour latent virus. Gammaherpesvirinae are growing in lymphocytes and can induce malignant transformation. Their host range is limited to family of the natural host. Some gammaherpesviruses also cause cytocidal infections in epithelial and fibroblastic cells. The virus can be maintained in latent form in lymphoid tissue. Transmission of herpesviruses is generally associated with close contact of skin and mucosal membranes, but droplet infection of the respiratory tract is also common. Many herpesviruses are host specific, but some alphaherpesviruses, such as pseudorabies virus and B virus (Herpesvirus simiae, cercopithecine herpesvirus 1), may affect a range of species. Most mammals, except primates and horses, are susceptible to the Pseudorabies virus (suid herpesvirus 1, PRV), which causes severe disease (Aujeszky disease, bulbar paralysis, ‘mad itch’). So far it is not certain that man is sensitive to PRV. In primates several species of the genus Macaca act as reservoir for the B virus. The transmissibility of B virus is limited, but it causes a fatal outcome in most cases of B-virus infection. B virus latency in man has been reported: a patient with encephalomyelitis and an ophtalmic zoster-like rash, and recurrent vesicular rash as a consequence of B virus infection. Man is the natural host for at least seven pathogenic herpesviruses: the two types of herpes simplex virus (HSV-1 and HSV-2), varicella-zoster virus (VZV), Epstein-Barr virus (EBV), human cytomegalovirus (HCMV), human herpesvirus-6 (HHV-6), and human herpesvirus 7 (HHV-7). Untill now, no clinical symptomatology has been linked with HHV-7 infection. The newly identified human herpesvirus in Kaposi’s sarcoma is called human herpesvirus 8 (HHV-8). In Table 1 are listed the names of the human herpesviruses according to the Sixth Report of
the International Committee on Taxonomy of Viruses (ICTV), and of the more recently discovered human herpesviruses.

How many more HHV’s as yet are undiscovered?

Table 1. *International and English vernacular names for human herpesviruses*

<table>
<thead>
<tr>
<th>FAMILY</th>
<th>TAXONOMIC STATUS</th>
<th>INTERNATIONAL NOMENCLATURE</th>
<th>ENGLISH VERNACULAR NAME</th>
<th>ABBREVIATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>HERPESVIRIDAE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subfamily Alphaherpesvirinae</td>
<td>Genus</td>
<td>Simplexvirus</td>
<td>Herpes simplexvirus group</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Species</td>
<td>Human herpesvirus 1</td>
<td>Herpes simplex virus 1</td>
<td>HSV-1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Human herpesvirus 2</td>
<td>Herpes simplex virus 2</td>
<td>HSV-2</td>
</tr>
<tr>
<td></td>
<td>Genus</td>
<td>Varicellovirus</td>
<td>Human herpesvirus 3</td>
<td>VZV</td>
</tr>
<tr>
<td></td>
<td>Species</td>
<td>Human herpesvirus 3</td>
<td>Varicella-zoster virus</td>
<td></td>
</tr>
<tr>
<td>Subfamily Betaherpesvirinae</td>
<td>Genus</td>
<td>Cytomegalovirus</td>
<td>Cytomegalovirus group</td>
<td>CMV</td>
</tr>
<tr>
<td></td>
<td>Species</td>
<td>Human herpesvirus 5</td>
<td>Human cytomegalovirus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Genus</td>
<td>Roseolovirus</td>
<td>Roseolovirus group</td>
<td>HHV-6</td>
</tr>
<tr>
<td></td>
<td>Species</td>
<td>Human herpesvirus 6</td>
<td>HHV-7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Human herpesvirus 7*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subfamily Gammaherpesvirinae</td>
<td>Genus</td>
<td>Lymphocryptovirus</td>
<td>Lymphoproliferativevirus group</td>
<td>EBV</td>
</tr>
<tr>
<td></td>
<td>Species</td>
<td>Human herpesvirus 4</td>
<td>Epstein-Barr virus</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Human herpesvirus 8*</td>
<td>Kaposi’s sarcoma-associated</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Genus</td>
<td>Rhadinovirus</td>
<td>Herpesvirus</td>
<td>(KSHV) HHV-8</td>
</tr>
</tbody>
</table>

* temporarily classified; not assigned in the Family of *Herpesviridae* by ICTV until now

1.2 Role of human herpesvirus infections in dermato-venereology

Human herpesvirus infections have a wide range of clinical manifestations. The severity of the disease depends on the entry site of the virus, the kind of infection (primary or recurrent), and the state of patient’s health (healthy, elderly or immunocompromised).
Alphaherpesviruses

In men and animals many alphaherpesviruses produce localized lesions, particularly of the mucosal membranes of the respiratory and genital tracts or the skin. Viral lesions appear in a specific order. Vesicles transform into pustules, which in turn become crusted or transform into superficial shallow ulcers covered by a pseudomembrane. The lesions usually heal without scar formation in less than fourteen days.\(^{2}\)

**Herpes simplex**

The human simplexviruses comprise human herpesvirus 1 (HSV-1) and human herpesvirus 2 (HSV-2). Man is the reservoir and vector of these herpesviruses. Wild and domestic animals are not affected. Herpes simplex viruses are among the most common infectious agents of man. Transmission is generally associated by close personal contact and droplet infection. They can cause a clinical manifest or asymptomatic infection.\(^{16}\)

The skin, oral cavity, genital mucosal membranes, conjunctiva and nervous system are the most frequent sites affected by HSV infection. However, in newborn infants, in immunocompromised and immunosuppressed patients other organ sites may be involved as well, as a consequence of viremia. Infection with HSV either during delivery (in case of genital herpes of the mother) or more often shortly thereafter by direct contact with persons with labial herpes ("kiss of death") or with herpetic whitlow poses a serious risk to the newborn and carries a risk of mortality.

Acute gingivostomatitis is the most common clinical manifestation of a primary herpes simplex virus type 1 infection. A primary HSV-1 infection of the mouth is sometimes accompanied with (primary) herpetic lesions on other body sites. Primary herpetic dermatitis, nasal herpes, ocular herpes, herpetic whitlow and genital herpes can be complications of acute gingivostomatitis. Recurrent herpetic lesions appear at or near the site of primary eruptions.\(^{16}\) The body sites commonly involved in human herpes simplex virus infection are listed in table 2.\(^{1}\)

The characteristic vesiculobullous eruptions of HSV can resemble those of other viral and non-viral skin infections.\(^{17-20}\) For instance impetigo caused by *streptococci* and *staphylococci*, and herpes simplex may have very similar clinical appearances. Eczema herpeticum, most commonly seen in patients with atopic dermatitis, but also reported in patients with Darier's disease, resembles secondary bacterial infected eczema.\(^{21}\)

Disseminated HSV infection can mimic varicella and dermatomal distribution of HSV lesions resembles herpes zoster. In case of intra-oral ulcers besides herpes simplex other ulcerous diseases, especially aphthous ulceration, must be considered. In patients with genital ulcers syphilis, ulcus molle, Behçet's syndrome, lymphogranuloma venereum, granuloma inguinale, candida infections, trauma, and toxic ulceration as side-effect in chemotherapy, must be considered in addition to HSV infection.\(^{16}\) Without the appearance of characteristic vesicles cutaneous HSV infection can be difficult to diagnose. Especially immunosuppressed patients often show atypical mucocutaneous manifestations of (persistent) HSV infection.\(^{22-24}\)

Erythema multiforme is considered to be a immunologic reaction to many exogenous agents, most commonly to recurrent herpes simplexvirus infections and drugs.\(^{25, 26}\)
Diagnostic aspects of human alphaherpesvirus infections in dermato-venereology

Table 2. Herpes simplex virus infections: commonly involved body sites and type of infection

<table>
<thead>
<tr>
<th>Involved sites</th>
<th>HSV type most usually found</th>
<th>Usual type of infection</th>
<th>Subclinical manifestation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mucous membranes:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>oral cavity</td>
<td>HSV-1</td>
<td>Primary</td>
<td>Frequently</td>
</tr>
<tr>
<td>lips</td>
<td>HSV-1</td>
<td>Recurrent</td>
<td></td>
</tr>
<tr>
<td>penis</td>
<td>HSV-2</td>
<td>Recurrent</td>
<td></td>
</tr>
<tr>
<td>vulva</td>
<td>HSV-2</td>
<td>Recurrent</td>
<td></td>
</tr>
<tr>
<td>vagina</td>
<td>HSV-2</td>
<td>Recurrent</td>
<td>Frequently</td>
</tr>
<tr>
<td>cervix</td>
<td>HSV-2</td>
<td>Recurrent</td>
<td>Frequently</td>
</tr>
<tr>
<td><strong>Eyes:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cornea</td>
<td>HSV-1</td>
<td>Recurrent</td>
<td>Frequently</td>
</tr>
<tr>
<td>conjunctiva</td>
<td>HSV-1</td>
<td>Primary</td>
<td>Frequently</td>
</tr>
<tr>
<td><strong>Skin:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>above the waist</td>
<td>HSV-1</td>
<td>Recurrent</td>
<td></td>
</tr>
<tr>
<td>below the waist</td>
<td>HSV-2</td>
<td>Recurrent</td>
<td></td>
</tr>
<tr>
<td>hands</td>
<td>HSV-1 &amp; 2</td>
<td>Recurrent</td>
<td></td>
</tr>
<tr>
<td><strong>Sensory ganglia:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>trigeminal</td>
<td>HSV-1</td>
<td>Recurrent</td>
<td></td>
</tr>
<tr>
<td>sacral</td>
<td>HSV-2</td>
<td>Recurrent</td>
<td></td>
</tr>
</tbody>
</table>

**Varicella-zoster**

The alphaherpesviruses VZV, pseudorabiesvirus (Suid herpesvirus 1), equine abortion virus (Equid herpesvirus 1), and simian varicella virus (Cercopithecine herpesvirus 9) are members of the genus Varicellovirus. Varicella-zoster virus (human herpesvirus 3, VZV) also belongs to the Alphaherpesvirinae subfamily. Man is the natural host of VZV, and is the etiological agent of varicella (chickenpox) and herpes zoster (shingles). Varicella is caused by primary infection and herpes zoster is the recurrent manifestation of VZV infection. Varicella is mainly transmitted by the respiratory route. The appearance of successive crops of skin lesions all over the body indicates a systemic infection with secondary intermittent viremia and the skin as the ultimate target organ. Primary VZV infection may involve any organ, mostly without clinical manifestation, except for the skin and occasionally the lung and brains. The initial skin lesions are macules and papules which rapidly become vesicular. The vesicles, surrounded by a small erythematous area, become rapidly crusted. The lesions heal usually within ten days, leaving sometimes small shallow scars. The lesions appear in successive crops so that all stages can be observed all over the body at any one time. The most common complication of varicella in otherwise healthy children is secondary bacterial infection of the lesions, which sometimes can lead to staphylococcal scalded skin syndrome (SSSS), and/or acute bacterial sepsis. Cerebellar ataxia, meningoencephalitis and encephalopathy (Reye's syndrome) may occur as neurologic
complication. Viral pneumonia in varicella can occur in previously healthy individuals, particularly in adults, but is mainly seen in immunocompromised patients. Recurrent varicella has been described in children infected with HIV. Varicella can be severe in pregnancy, and poses a high risk of complications to the mother and the fetus. VZV infection contracted before the 20th week of pregnancy can cause the rare congenital varicella syndrome of the baby, with scarring of the skin, musculoskeletal defects, cerebral and ocular damage. VZV is known to cross the placenta very late in pregnancy. If the mother contracts varicella within a week before the baby's birth, or if the child is infected before it is one week old, the infant may suffer from neonatal varicella.(27, 28)

Herpes zoster results from the reactivation of latent varicella-zoster virus in a single sensory ganglion. It typically affects a single dermatome of the skin and the sitting of the skin lesions depends on the involved ganglion. Especially in immunocompromised patients the virus can spread from the affected dermatome to infect the skin or other organ at some distal site, and in case of extensive dissemination the appearance can resemble varicella.(29)

In most patients a VZV infection is diagnosed from the typical clinical features. Clinical diagnosis of herpes zoster in immunocompromised patients can be difficult, especially in the early stages of the disease, when only a few herpetic-like vesicles are present. These may not have the typical dermatomal localization.(30) In addition, due to acyclovir resistant VZV patients with acquired immunodeficiency syndrome may develop unusual cutaneous patterns like disseminated ecthymatous lesions,(31, 32) hyperkeratotic nodules an crusted verrucous lesions,(33-38) pox-like lesions,(39) and disseminated pinpoint-sized erythematous papules.(40) Zoster-like cutaneous manifestations can develop in other diseases giving cause to faulty clinical VZV-diagnosis.(41-43)

Several types of cutaneous lesions have been reported at the sites of resolved cutaneous herpes zoster lesions. These comprise comedones, xanthomatous changes, sarcoidal and tuberculoid granulomas, granulomatous vasculitis and unclassified granulomaous dermatitis, nodular solar degeneration, pseudolymphoma, psoriasis, lichen planus, morphoea, lichenoid chronic graft-versus-host disease, eosinophilic dermatosis, acquired reactive perforating collagensis, lymphoma, leukemia, Kaposi's sarcoma, angiosarcoma, basal cell and squamous cell carcinoma, and cutaneous metastases from internal carcinoma.(44)

In immunosuppressed patients chronic lichenoid dermatoses may be associated with a chronic, non-cytolytic, low-productive VZV infection.(24, 45)

**Betaherpesviruses**

**Cytomegalovirus**

Human cytomegalovirus (human herpesvirus 5, CMV) belongs to the Betaherpesvirinae subfamily. Primary infection with CMV can be acquired intrauterine transplacentally especially from the primary infected mother. Perinatal infection is acquired mainly from infected maternal secretions or breast milk. The postnatal routes of transmission of CMV infection are salivary transmission, blood transfusion, and organ transplantation. Urine is an additional source of infection in children. Semen may contain virus so that it can be spread via sexual contact. The incubation period of CMV is unknown, but is estimated to
be four to eight weeks. After a clinically silent infection of unknown host cells probably at the site of infection, for example the upper respiratory tract, the virus spreads locally to lymphoid tissues and then systemically infects circulating lymphocytes and monocytes to involve lymph nodes and the spleen. The infection then localizes in epithelial cells in salivary glands, kidney tubules, cervix, tests and epididymis, from whence virus shedding occurs. In infected individuals CMV has been demonstrated in tissues of epithelial origin (kidney, liver, bile ducts, salivary glands, gut, lung, pancreas) as well as in endothelial cells. In vitro CMV replication is only possible in human fibroblasts. More than 90% of congenital infected babies have no symptoms at birth. The classic symptoms at birth are intra uterine growth retardation, jaundice, hepatosplenomegaly, trombocytopenia and central nervous system involvement. Purpura can be present due to the trombocytopenia. Other complications are pneumonitis and myocarditis. Perinatal and postnatal CMV infection is mostly clinically not apparent. Also, recurrences are in general mostly asymptomatic.

In immunocompromised and AIDS patients CMV disease is a more serious threat. CMV infection, usually as reactivation of latent virus, is one of the AIDS-defining opportunistic infections. Retinitis in patients with AIDS is the most frequent manifestation of CMV infection, which can also cause gastrointestinal disease, hepatitis, encephalopathy and pneumonia. Skin lesions caused by CMV are not frequently seen in these patients. Vasculitis, hyperpigmented nodules and plaques, papular exanthema, warty hyperkeratotic lesions, and (perineal) ulcers are reported. Concurrent skin infections of HSV and VZV with CMV are described, but these are probably due to CMV viremia without specific cutaneous involvement.

Human herpesvirus 6
Human herpesvirus 6 (HHV-6) has two variants, A or B (HHV-6A and HHV-6B). This virus is a mature T-cell lymphotropic virus and shares genetic homology with human cytomegalovirus. HHV-6B is the causative agent for exanthema subitum (roseola infantum). However, the etiologic role for HHV-6A has not yet been clarified. The full clinical spectrum of diseases caused by HHV-6 infection has not yet been confirmed. Because of the high incidence of infection in childhood primary HHV-6 infection of adults is rare. HHV-6 infection in adults can lead to hepatitis, and mononucleosis-like illness with prolonged lymphadenopathy. The characteristic symptoms of exanthema subitum (ES) are prodromal high fever persisting 3-5 days, followed by the disappearance of the general symptoms and the onset of a maculopapular rash, that lasts 1–2 days. The rash is generally localized on the trunk and the neck, but also on the lower part of the face and the extremities. This syndrome of fever, defervescence, and rash occurring in infants is not always caused by HHV-6, but also occurs in infections such as with HHV-7 and enteroviruses.

Human herpesvirus 7
In 1990 another human herpesvirus, HHV-7, has been isolated, from CD-4 positive T-cells. Infection, as determined by seroconversion, occurs in most children, but at later age than in the case of HHV-6. The virus persists, and is present in the majority of normal adult saliva samples. The role of HHV-7 in human disease is unclear.
co-factor in HHV-6 related syndromes. The primary infection with HHV-7 is linked to febrile illness with or without rash that resembles ES. Association between reactivation of HHV-7 infection and pityriasis rosea has recently been published. Interaction among HHV-7 and EBV in mononucleosis syndrome has been suggested.

**Gammaherpesviruses**

*Epstein-Barr virus*

Epstein-Barr virus (human herpesvirus 4, EBV) belongs to the *Gammaherpesvirinae*. EBV infects squamous epithelial cells and B lymphocytes where it remains latent after primary infection. Exposure to EBV is often asymptomatic, and is only apparent from seroconversion in most individuals. However, primary infection may be symptomatic. The most common presentation of primary EBV infection is infectious mononucleosis (IM) in adolescents. Up to 10% of patients with IM may develop dermatologic manifestations: jaundice, maculopapular exanthema (especially related with penicillin and ampicillin administration), palatal petechiae and cutaneous hemorrhage (in cases with marked trombocytopenia), and occasionally urticarial eruptions. Recurrent infection by reactivation of latent virus is seen in immunocompromised patients, resulting in recurring chronic fevers, weight loss, lymphadenopathy, hepatosplenomegaly, interstitial pneumonia and uveitis. In patients with HIV infection a distinctive lesion in the mouth is known as oral hairy leukoplakia.

In Africa EBV is associated with Burkitt's lymphoma in areas where malaria is endemic. In certain areas of China, South-East Asia and North Africa, EBV is associated with nasopharyngeal carcinoma, probably due to genetical predisposition of the population. Lymphoproliferative disorders such as Hodgkin's disease, non-Hodgkin's lymphoma may result from EBV infection in immunocompromised patients and males with the X-linked lymphoproliferative syndrome. A case of chronic bullous disease of childhood following EBV seroconversion has been reported.

*Human herpesvirus 8*

The newly identified human herpesvirus 8 (HHV-8) associated with Kaposi's sarcoma shares limited homology with EBV. HHV-8 resembles EBV in that it has a tropism for epithelial and B-cells, it is kept under immunological control, and only presents a problem during immunosuppression. An AIDS–related body cavity–based lymphoma has recently been linked to HHV-8.

**Summary**

Dermatologic manifestations caused by human herpesviruses are listed in table 3.
Diagnostic aspects of human alphaherpesvirus infections in dermato-venereology

Table 3. Dermatologic manifestations of human herpesvirus infections

<table>
<thead>
<tr>
<th>VIRUS TYPE</th>
<th>TYPE OF DISORDER</th>
</tr>
</thead>
<tbody>
<tr>
<td>HHV1 (HSV1)</td>
<td>mucocutaneous lesions (gingivostomatitis, cold sores); ophtalmic infection (keratoconjunctivitis).</td>
</tr>
<tr>
<td>HHV2 (HSV2)</td>
<td>mucocutaneous lesions (ano-genital herpes, cutaneous herpes);</td>
</tr>
<tr>
<td>HHV3 (VZV)</td>
<td>varicella (chickenpox), zoster (shingles);</td>
</tr>
<tr>
<td>HHV4 (EBV)</td>
<td>ophtalmic infection (ophtalmic zoster).</td>
</tr>
<tr>
<td>HHV5 (CMV)</td>
<td>maculopapular rash (especially related to antibiotic administration), facial oedema; oral hairy leukoplakia (immunocompromised host).</td>
</tr>
<tr>
<td>HHV-6A</td>
<td>purpura (congenital infection) / vasculitis, hyperpigmented nodules and plaques, papular exanthema, warty hyperkeratotic lesions, and (perineal) ulcers (immunocompromised host).</td>
</tr>
<tr>
<td>HHV-6B</td>
<td>a clear etiologic role has not yet been identified.</td>
</tr>
<tr>
<td>HHV7</td>
<td>exanthema subitum; in adults: hepatitis, mononucleosis-like illness.</td>
</tr>
<tr>
<td>HHV8</td>
<td>mononucleosis-like illness (interaction among other human herpesviruses?);</td>
</tr>
<tr>
<td>HHV8</td>
<td>Kaposi’s sarcoma; primary effusion lymphoma (body cavity-based lymphoma).</td>
</tr>
</tbody>
</table>

1.3 Microbiology of herpetic infection

All members of the Herpesviridae have an identical morphology of the virus particle (virion). By negative stain transmission electron microscopy the herpesviruses show a pleomorphic appearance, but this is artificially induced during processing. Pseudo-replica electron microscopy reveals the most accurate representation of the virus particle. The overall diameter of the complete enveloped herpesvirus particle is between 120 and 300 nmm and depends upon the particular virustype.\(^3,5\)

The herpesvirus has four basic structures: 1.) an electron opaque core, 2.) an icosadeltahedral capsid enclosing the core, 3.) an amorphous electron-dense material (tegument) surrounding the capsid, and 4.) an outer membrane (envelope) which surrounds the nucleocapsid and tegument. The herpesvirus envelope contains numerous glycoprotein protrusions (spikes), which are important antigenic determinants of the herpesviruses. The herpesvirus core is composed of linear double-stranded DNA packaged in the form of a torus.\(^3,5\)

The various herpesviruses cannot be differentiated on the basis of the virion morphology. The general features of their replicative cycle is basically also the same. To initiate infection, the virus must attach to cell receptors. Initial attachment and penetration of the host cell is mediated by the glycoprotein spikes on the surface of the envelope. After apposition of the virion envelope and the host cell plasma membrane fusion occurs, which results in the introduction of tegument proteins and nucleocapsid into the host cell cytoplasm.
Tegument proteins induce a decrease of host DNA synthesis, protein synthesis, ribosomal RNA synthesis and glycosylation of host proteins. The nucleocapsid is transported through the cytoplasm to a nuclear pore. At the nuclear pore the capsid degrades and viral DNA is released into the cell nucleus. The transcription of viral DNA takes place in the nucleus. Mature nucleocapsids bud through the nuclear membrane and acquire their tegument and envelope. By electron microscopy the virus envelope shows a typical trilaminar appearance and it seems to be derived from patches of altered nuclear membranes, modified by insertion of virus glycoprotein spikes. The virion egresses from the infected cell by transit through the cisternae of the rough endoplasmatic reticulum of the Golgi apparatus and via cytoplasmatic transport vesicles. Viral replication finally results in the destruction of the host cell.\textsuperscript{16, 78}

In cells with active herpesvirus replication typical intranuclear inclusions are formed, representing crystalline arrays of virus particles. These Lipschütz (Cowdry type A) intranuclear inclusion bodies are characteristic for the cell pathology all herpesvirus infections, but in histopathological examination they cannot be used to differentiate the human herpesviruses from each other. Herpesviruses induce fusion of the cells they have infected. This will generate multinucleated giant cells (polykaryocytes) that can be demonstrated in affected tissues by direct light microscopic examination.\textsuperscript{16, 78}

Alphaherpesvirinae can establish latent infection. The site of virus latency is related to the site of primary infection. During the primary infection epithelial receptors of local sensory nerves in the affected skin or mucous membranes come into contact with the virus, which penetrates the nerve and moves by axoplasmatic flow within the nerve to the perikaryon. The pathogenetic mechanism in herpesvirus latency, and the mechanism of reactivation of latent virus in recurrent infection, has not been solved yet. The virus egress from the latently infected sensory nerve and infects adjacent epithelial cells causing recurrent infection.\textsuperscript{16, 78}

At present it is not yet clear that a recurrent human cytomegalovirus infection is caused by reactivation of latent virus, or by reinfection.\textsuperscript{46}

The Epstein-Barr virus probably induces latently infected long-living memory B-lymphocytes.\textsuperscript{71, 79}

1.4 Methodology for diagnosing herpetic infections

Cleator stated that “To achieve meaningful diagnosis a close collaboration between clinic and laboratory is necessary”.\textsuperscript{16}

As with all viruses, there are two potential strategies for providing a laboratory diagnosis: detection of virus or the demonstration of a specific immune response. The aim of this chapter is to provide a general overview rather than a complete, indepth analysis of diagnostic methods. Each test has advantages and disadvantages.
Diagnosis of HSV infection

The diagnosis of mucocutaneous manifestations of HSV infection is predominantly clinical. Laboratory diagnosis may be required to confirm the clinical diagnosis, to look for asymptomatic viral shedding, and to demonstrate HSV infection in HSV-atypical lesions in the immunocompromised patient.

In HSV and VZV infection, the virus-infected cells are located in the base of the herpes lesions and the virus is released into the vesicle. The roof of the vesicle will not contain virus, and, therefore, this material is not suitable as specimen for herpes diagnosis. The appropriate specimen should be taken from the vesicular fluid and the infected cells from the base of the lesion. Virologic methods for HSV diagnosis include direct demonstration techniques (light and electron microscopy), detection of viral antigens in specimens by immunoassays (immunofluorescence cytology, immunosorbent immunoassays), virus culture, detection of viral DNA (hybridization techniques, polymerase chain reaction) and serology. These methods are summarized below.

Direct demonstrating techniques

*Light microscopy*

Source: cells derived from mucocutaneous papulovesicular, vesicular, bullous, pustular, and ulcerous lesions, scraped from the base of the lesion (*Tzanck smear*); tissue biopsy and necropsy specimens.

Detection: multinucleated giant cells; intranuclear inclusion bodies.

Sensitivity: acceptable only with typical herpetiform lesions, high sensitivity in vesicular stage (subject of study in this thesis).

Advantage: rapid assay (minutes), easy to perform, inexpensive and suitable for office diagnosis.

Disadvantage: cannot differentiate between HSV-1, HSV-2 and VZV; inadequate sensitivity for cervical lesions and asymptomatic shedding.

*Electron microscopy*

Source: vesicle fluid; cells derived from papulovesicular, vesicular, bullous, pustular, and ulcerous lesions, scraped from the base of the lesion; crusted lesions; biopsy and necropsy specimens; serum (detection of serum HSV antibodies, subject of study in this thesis).

Detection: herpesvirus particles, and herpesvirus induced antibodies.

Sensitivity: relatively insensitive assay (a specimen must contain at least 106 particles pro millilitre); sensitivity increases by ultracentrifugation methods and immunological capture assays; acceptable only with typical herpetiform lesions; highest sensitivity in the vesicular stage. (Subject of study in this thesis).

Advantage: all type of lesions can be used for virus detection (subject of study in this thesis); rapid with standard negative staining technique (within an hour of receipt of the specimen in the laboratory), and several hours with immune
EM techniques to type HSV and VZV virus particles; serology by EM can be combined with virus detection in the same EM session (subject of study in this thesis).

Disadvantage: not widely available; laborious; expensive.

**Immunoassays**

*Immunofluorescence microscopy*

Source: cells derived from papulovesicular, vesicular, bullous, pustular, and ulcerous lesions, scraped from the base of the lesion; tissue biopsy and necropsy specimens.

Detection: viral antigens.

Sensitivity: sensitive assay, comparative with virus culture.

Advantage: rapid results (few hours), specific detection of HSV, low cost.

Disadvantage: less sensitive for cervical lesions and asymptomatic shedding.

*Immunosorbent immunoassays*

Source: herpes simplexvirus-infected cells.

Detection: viral antigens.

Sensitivity: sensitive assay; radio- and enzyme-immunoassays achieve not the same sensitivity as virus culture for infectious virus.

Advantage: rapid in comparison with virus culture; can detect non-infectious virus, and viral antigens.

Disadvantage: use of radioisotopes in radio-immunoassays.

**Virus culture**

Source: vesicle fluid, swabs or scrapings from the base of the lesion, saliva, urine, cerebro-spinal fluid (CFS), tissue biopsy and necropsy specimens. Sensitivity of virus culture depends upon the preservation of virus infectivity within the clinical specimen. To reduce loss of infectivity during transportation to the laboratory a suitable transport medium is needed. If transportation takes more time and/or temporary storage is needed, the material should be maintained at +4°C; in case of prolonged/final storage immersion in liquid nitrogen (at -196°C), or, when not possible, at least kept at -70°C. CFS, biopsy and necropsy specimens should be transported in dry sterile containers and maintained at +4°C.

Detection: infectious viruses.

Sensitivity: high sensitivity, with a theoretical detection limit of one infectious particle (golden standard), but not as sensitive as was believed on theoretical grounds; sensitivity can be increased by centrifugational methods.

Advantage: allows virus typing and drug sensitivity testing.

Disadvantage: virus is rarely isolated from crusted lesions.
Viral nucleic acid detection

Hybridization techniques
Source: biopsy or necropsy material (in situ hybridization); clinical samples other than tissue sections (dot-blot hybridization).
Detection: viral DNA.
Sensitivity: sensitive assay.
Advantage: rapid compared to virus culture.
Disadvantage: expensive.

Polymerase chain reaction
(PCR subject of study in this thesis)
Source: all clinical samples.
Detection: viral DNA.
Sensitivity: highly sensitive detection.
Advantage: rapid compared to virus culture.
Disadvantage: false positive reactions by external contaminant DNA; false negative results in cerebrospinal fluid by contamination of hemoglobin or the presence of inhibiting factors; expensive (special laboratory, logistics, specialists).

Serology

There is no standard serological technique for HSV diagnosis. Commonly used techniques are ELISA (enzyme-linked immunosorbent assay), CFT (complement fixation test), Western-blot and immunodot techniques.

Source: serum (to demonstrate intrathecal synthesis of HSV antibodies in case of central-nervous-system infection HSV IgG antibody and albumin in serum are compared with the titers in the cerebrospinal fluid).
Detection: HSV-specific IgA, IgG, and IgM class antibodies.
Sensitivity: high, except in recent infection; depends largely on the applied method (CF is relatively insensitive).
Advantage: useful in epidemiological seroprevalence studies; investigation of paired sera demonstrates recent acquired infection. (EM serological diagnosis is subject of study in this thesis)
Disadvantage: IgM antibody response does not allow reliable differentiation of primary infection and virus reactivation, except for neonatal HSV infection.
Diagnosis of VZV infection²⁷, ²⁸, ⁸³-⁸⁸)

Varicella and herpes zoster are usually diagnosed from the clinical picture. Laboratory diagnosis is generally required when there is doubt about the clinical diagnosis, especially important in case of atypical lesions in immunocompromised patients. Confirmation of VZV infection is also needed for the treatment of patients at risk of contracting the severe forms of VZV infection, and for treatment in pregnancy.

Virological methods for VZV diagnosis are about the same as for HSV: demonstration of specific cytological changes in herpesvirus-infected cells, detection of viral proteins in specimens using immunologic reagents, virus culture, detection of viral DNA (e.g. by dot-blot, in situ hybridization, and polymerase chain reaction), demonstration of herpesvirus particles by EM and virus typing combined with immunological techniques, and serological diagnosis (immuno-EM is subject of study in this thesis).

Virus culture of VZV is less sensitive than HSV virus culture, mainly due to temperature inactivation of VZV during storage and transportation to the laboratory. Samples not inoculated shortly after sampling will not yield good growth of virus. The VZV particle is degraded by prolonged storage at temperatures of -10°C or above. If transportation and/or storage takes more than a few hours, the specimen should be kept on dry ice, or immersed in liquid nitrogen (or, when not possible, at least kept at -70°C). To obtain optimal results from virus culture, the specimen should be inoculated as soon as possible.

VZV grows slowly compared to HSV. The average time for VZV isolation from clinical specimens is 7 days. Therefore, VZV culture is not suitable for rapid confirmation of clinical diagnosis. Before cytopathic effect becomes apparent, it is possible to detect VZV viral antigens with monoclonal antibodies. This can reduce the time to diagnose VZV infection considerably. Positive HSV and VZV cultures differ in growth characteristics, cell line sensitivity and the virus specific cytopathological effect. For additional prove monoclonal antibodies are now commonly used.

Diagnosis of human cytomegalovirus infection⁴⁶, ⁴⁷)

Congenital or neonatal CMV infection must be differentiated from toxoplasmosis, rubella, herpes simplex and varicela-zoster virus infection. CMV infection in the immunocompromised host is severe and frequently life-threatening. To reduce the incidence of CMV infection following organ transplantation, the donor and recipient must be screened for their CMV status.
The following laboratory tests can be used for detection of CMV infection:

**Direct demonstration techniques**

*Light microscopy*
- **Source:** tissue specimens.
- **Detection:** swollen CMV infected cells have large nuclei containing intranuclear inclusions with the characteristic 'owl's eye' aspect.
- **Sensitivity:** not sensitive.
- **Advantage:** assessment of CMV infection in diseased organs (detection of viraemia cannot prove this).

*Electron microscopy*
- **Source:** urine (samples from congenitally infected infants usually contain high titers of CMV, not detected in urine samples from adults).
- **Detection:** herpesvirus particles.
- **Sensitivity:** sensitive in case of infected infants.
- **Advantage:** rapid assay (which only takes a few hours). Specific in case of infected infants (no other herpesvirus causes such high titers in urine from infants).
- **Disadvantage:** genital herpes, frequently seen in patients with AIDS, can cause viral shedding in urine. With negative staining no discrimination is possible between the human herpesviruses; immune EM can differentiate between HSV and CMV virus.

**Immunassays**
- **Source:** tissue specimens, bronchoalveolar lavage fluid.
- **Detection:** CMV infected cells; early viral antigens by leucocyte antigen detection.
- **Sensitivity:** high.
- **Advantage:** rapid assay.

**Virus culture**
- **Source:** urine, saliva, unseparated heparinized blood, tissue specimens. Samples should be send fresh to the laboratory as soon as possible (or on wet ice without freezing, if a delay of more than a few hours is expected).
- **Detection:** infectious cytomegalovirus; rapid diagnosis by detection of early antigen fluorescent foci (DEAFF).
- **Sensitivity:** high.
Viral nucleic acid detection

*Polymerase chain reaction*

**Source:** all clinical samples.

**Detection:** viral DNA

**Sensitivity:** very sensitive.

**Advantage:** rapid assay compared to virus culture.

**Disadvantage:** detection of clinically not relevant low virus quantities and latent virus (without prognostic value). False negative results by interference of unknown tissue factors can occur.

Serology

Many laboratory techniques have been described for the detection of CMV induced antibodies.

**Source:** serum.

**Detection:** CMV specific IgG and IgM class antibodies. IgM antibodies cannot be found in immunocompetent patients with a recurrent infection. Renal transplant patients can produce IgM antibodies during recurrent infection.

Immunocompromised patients with a primary infection sometimes fail to produce specific IgM antibodies. Seroconversion of specific IgG antibodies points to an acute infection. Rising titers of IgG antibody without seroconversion may be caused by a primary or recurrent infection. Increase of both CMV specific IgG and IgM antibodies can occur in bone marrow transplant recipients, but this not necessarily means that the patient will get the disease.

**Sensitivity:** depends on the method in use.

**Disadvantage:** the rheumatoid factor interferes with detection of CMV-specific IgM antibodies.

Diagnosis of epstein-barr virus infection

The diagnosis of infectious mononucleosis (IM) is based on the clinical picture, supported by the presence of ‘atypical’ blood lymphocytes, and confirmed by serology. The heterophil antibody test (e.g. Paul–Bunnell test) is the test of choice for diagnosis of IM and detects an antibody which causes haemagglutination of non-human erythrocytes. Specific EBV antibodies to viral capid antigens (IgM VCA and IgG VCA) and Epstein-Barr nuclear antigens (EBNA) can be detected, and, except for IgM VCA, persist lifelong. Early antigen antibodies to EBV (Anti-D and Anti-R) persist from several months up to several years. Specific antibodies to soluble complement-fixing antigens (Anti-S) and neutralizing antibodies also persist lifelong. Determination of EBNA and Anti-S antibodies may be helpful in diagnosis in cases with a negative heterophil antibody test.
Diagnosis of human herpesvirus type 6 infection\textsuperscript{(11, 50)}

The diagnosis of exanthema subitum is primarily clinical. HHV-6 can be identified by virus culture, viral antigen detection, and in the convalescent phase of exanthema subitum by antibody detection. Virus isolation is attempted from peripheral blood of patients with manifest exanthema subitum, and from blood of severely immunocompromised immunocompromised patients.

Diagnosis of human herpesvirus type 7 infection\textsuperscript{(10, 90, 91)}

Virus isolation, serology, PCR, and electron microscopy have been used to detect HHV-7 infection. The role of HHV-7 in human disease is still unclear. However, some investigators claim the etiologic role of HHV-7 in pityriasis rosea\textsuperscript{(67-69)}.

Diagnosis of human herpesvirus type 8 infection

Diagnosis of HHV-8 related diseases has not yet passed the research state.\textsuperscript{(13)}
1.5 Aim and outline of this thesis

The diagnosis of varicella, herpes zoster and herpes simplex can be made on the basis of physical examination and clinical history. Because vesiculobullous eruptions of other viral and nonviral skin infections can resemble those of HSV and VZV, the infection can be misdiagnosed, if not confirmed by laboratory tests. This can be a hazardous situation for some group of patients. A rapid conclusive diagnosis is very important for adequate antiviral therapy. Delay in treatment will diminish the effectiveness of the antiviral drugs. In many hospitals in Holland an adequate laboratory facility for viral culture is not available. This is also the case for more sophisticated viral diagnostic techniques, like PCR. Specimens must be sent to specialized virological laboratories elsewhere, and therefore the processing of viral specimens will be delayed. Especially VZV can lose infectivity very rapidly when kept in transport medium for longer periods. Even under optimal laboratory conditions, VZV isolation is substantially less sensitive than those for HSV.

The principal aim of this thesis is to inform the dermato-venereologist on expectations and pitfalls of diagnostic methods available for human alphaherpesviruses. This was done by applying old and newly developed methods on well described clinical samples (Chapter 2, 3, and 4). This thesis also aims to get more insight into the virus induced immuneresponses taken place at the level of the skin, and how these processes and serum antibody responses cohere (Chapter 5).

Chapter 2.1 describes a rapid diagnostic test (Tzanck test) to exclude herpesvirus infections in vesicles, blisters and pustules, especially useful for office diagnosis. The diagnostic value of this test in herpetic and non-herpetic vesicular and bullous skin disorders in pediatric practice is evaluated in chapter 2.2. The value of the Tzanck smear in comparison with virus culture in diagnosis of anogenital lesions suspected of HSV infection is described in Chapter 2.3.

Chapter 2.4 emphasises the need for investigating every neonate with pustules to exclude herpetic infections. A systematic diagnostic approach of pustular eruptions in the neonate is proposed.

Electron microscopy can be applied as a rapid method for virus diagnosis, and can be used in validation studies. Chapter 3.1 reports on colloidal gold immunoelectron microscopy for rapid diagnosis of VZV infections by discrimination between VZV, HSV-1 and HSV-2. Chapter 3.2 describes improved methods for detection of HSV by electron microscopy in clinical specimens. Chapter 3.3 outlines the sensitivity and specificity of the Tzanck preparation in comparison with viral culture and electron microscopy. Chapter 3.4 reports on a case of human T-cell lymphotrophic virus type 1-positive leukemia complicated by atypical multidermatomal herpes zoster. Where standard tests failed, cytodiagnosis (Tzanck test), and immuno-electron microscopy unmistakably have proven VZV infection. As addendum to chapter 3 we describe an easy method of storage of EM-grids, applied to this study.

The continuous need for more specific and sensitive diagnostic methods lead to the introduction of polymerase chain reaction (PCR) in HSV and VZV diagnosis. In chapter 4 PCR for VZV, based on degenerative primers, was used to diagnose clinical specimens. Because degenerated primers were used in PCR, glycoprotein B DNA could be amplified
Diagnostic aspects of human alphaherpesvirus infections in dermato-venereology

from all alphaherpesvirus field strains, present in clinical samples. The amplification of glycoprotein B allowed virus typing of VZV, HSV-1 and HSV-2 using restriction enzyme digestion on the PCR-products.

Human immunologic responses to HSV and VZV infections comprise humoral and cell mediated immunity, together with other nonspecific host responses. VZV immune serum globulin prophylaxis, still applied by high-risk patients, reduces the attack rate and the severity of primary VZV infection. How this is reached is still not clear. Anti-VZV immunoglobulins (VZIG) play a role in the lysis of infected cells by antibody-mediated cellular cytotoxicity. It is still not known whether VZIG can abort viral replication at the initial sites of infection (i.e. the regional lymph nodes) during a primary VZV infection, can protect against further spread of the infection via the blood (viraemia), or is effective at both levels of infection. Serologic procedures, although giving an incomplete picture of immunity to VZV, are used to assess the immune status of the patient. However, serological cross-reactivity between HSV and VZV may cause a false positive outcome of serological test. Chapter 5 describes studies on serologic cross reactions between VZV and HSV. The results of serology by IEM are compared with those obtained by techniques employing fluorescent antibody to membrane antigen (FAMA), carried out on infected cell monolayers. The influx of T- and B-lymphocytes, and the presence of complement factors in herpetic skin lesions, studied with immunohistology, are compared with the presence of virus and the appearance of virus-immune complexes in vivo in relation to anti-VZV serum antibody titers. Activation of immune effector mechanisms against VZV and differences in the immune response in varicella versus herpes zoster are discussed.

In chapter 6, the findings of this thesis are summarized and discussed.
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