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Review

Introduction: Is viral shedding a surrogate marker for transmission of genital herpes?

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Abstract

Genital herpes, caused by either herpes simplex virus type 1 or 2 (HSV-1 and HSV-2), is a significant public health problem worldwide. It increases the risk of infection with HIV, upregulates HIV after infection and can be associated with serious morbidity and mortality. It is now known that clinical and subclinical viral reactivation with resultant shedding from anogenital mucosa occurs frequently, resulting in transmission during sexual contact. Sexual transmission of HSV infection is common, even between monogamous individuals. Antiviral therapy reduces the frequency and degree of viral shedding and lowers the transmission rate in discordant monogamous couples, although transmission can still occur in people prescribed antiviral therapy. These encouraging data raise important questions for the management of genital HSV infection, particularly with regard to the prevention of transmission. Although the quantity of virus present is clearly important in transmission of some viruses, it is not clear whether this is the case for HSV transmission. Ideally, a surrogate marker needs to be able to identify individuals with detectable amounts of virus, and differentiate them from individuals with detectable amounts of virus that are transmissible. The aim of this supplement is to explore the issues surrounding the validation of surrogate markers of transmission of HSV, using examples from other human viral diseases, and to review the available evidence. In the future, exploration of these issues may shed light on management and prevention strategies. In particular, the results may clarify what evidence is required to warrant prescribing a drug for reducing HSV transmission, and for which patient populations this strategy is appropriate.

Keywords: HSV-2; Shedding; Transmission; Pathogenesis; Surrogate

1. Introduction

Genital herpes is caused by either herpes simplex virus type 1 or 2 (HSV-2 and HSV-1) and is a significant public health problem worldwide. Aside from the direct consequences of life-long viral infection, HSV increases the risk of infection with HIV and up-regulates HIV after infection. Furthermore, the complications of herpes can be significant, and in the case of neonatal HSV infection, can be associated with serious morbidity and mortality.

Clinical and sub-clinical viral reactivation with resultant shedding from anogenital mucosa occurs frequently, particularly for HSV-2 infection, resulting in transmission during sexual contact. During periods of asymptomatic shedding, an infected person is likely to be unaware of this increased risk associated with viral shedding and consequently sexual transmission of HSV infection is common, even between monogamous individuals. Indeed, daily antiviral therapy reduces the frequency and degree of viral shedding (Wald

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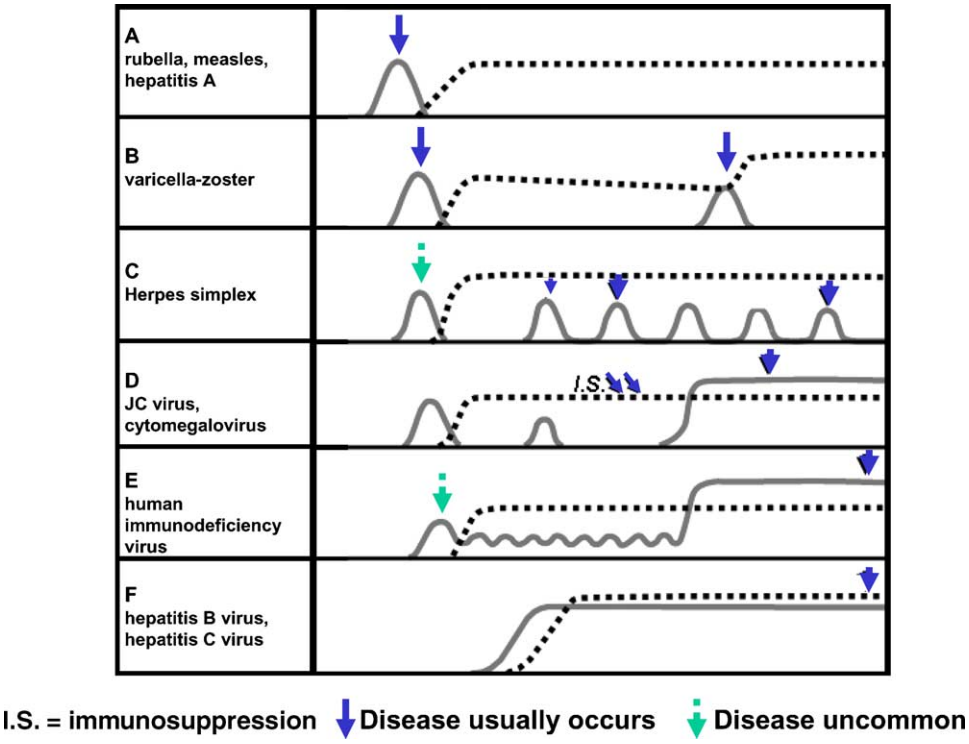

Fig. 1. Patterns of viral replication.

et al., 1996, 1997; Sacks et al., 1997) and lowers the transmission rate in discordant monogamous couples (Corey et al., 2004). However, transmission can still occur in people prescribed antiviral therapy (Corey et al., 2004). These encouraging data raise important questions for the management of genital HSV infection, particularly with regard to the prevention of transmission. For example, is transmission of HSV linked directly with viral shedding and, if so, what quantity of virus is needed and over what duration? Will a reduction of HSV shedding always lower the transmission rate? Is the reduction in transmission directly proportional to decreased shedding? Is viral shedding a surrogate marker for transmission in HSV?

The aim of this supplement is to explore the issues surrounding the validation of surrogate markers of transmission of HSV, using examples from other human viral diseases, and to review the available evidence. In the future, the exploration of these issues may shed light on management and prevention strategies. In particular, the results may clarify what evidence is required to warrant prescribing a drug for reducing HSV transmission and for which patient populations.

2. Pathogenesis

The first question for consideration is how pathogenesis and changes in the magnitude of virus shed within an individual relate to transmission of HSV between persons.

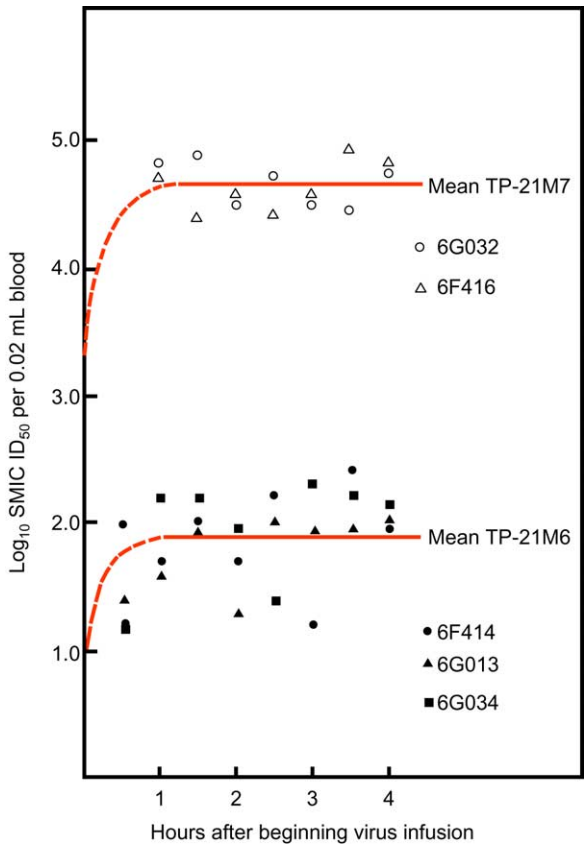

Fig. 2. Turnover of circulating virus. Reprinted from Nathanson and Harrington (1967) with permission from the Oxford University Press.

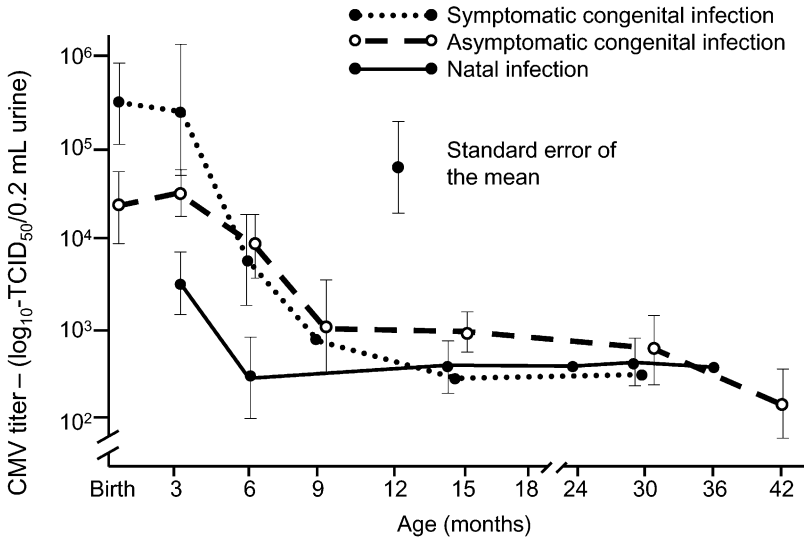

Fig. 3. Viral load is greater in children with overt CMV disease after congenital infection. Reprinted from Stagno et al. (1975) with permission from the University of Chicago Press.

Different viruses cause disease in different ways, and various patterns of viral pathogenesis are shown in Fig. 1. The solid line represents the virus, the dotted line indicates host immunity, the small solid arrows show symptoms, and the large broken arrows indicate where disease is uncommon. These different patterns illustrate that viral load markers have a complex relationship with pathogenesis and transmission.

Many viruses, including rubella and measles, cause acute infections (panel A). Symptoms occur at the peak of viral infection or soon after, and the infected person either dies or recovers. The viral infection elicits an immune response which clears the infection, leading to recovery, after which the person demonstrates immune memory for that virus. In terms of transmission, the virus has only one chance to be passed to another susceptible host. Panel B shows that varicella zoster virus (VZV) has two opportunities for transmission: the host is infectious during the initial infection (varicella), and the virus often reactivates (usually only once) in later life when immunity wanes causing herpes zoster. Herpes simplex virus (panel C) generally recurs many times during the lifetime of an infected individual. While some recurrences are symptomatic and others are not, all episodes can lead to transmission. Cytomegalovirus (CMV) is also prevalent in the community and is often transmitted without causing any symptoms, only leading to serious problems if the host becomes immunosuppressed, for example by the drugs needed for allotransplantation (panel D). In such circumstances or in the newborn, the viral load increases until a threshold is passed, at which point disease occurs. Infection with HIV (panel E) also produces disease at high viral loads, but in this case it is the virus itself that causes immunosuppression. Panel F shows that hepatitis B and C viruses usually give no initial symptoms, but damage the liver progressively so that disease does not manifest until many decades later.

In a classic demonstration of viral pathogenesis, Nathanson and Harrington (1967) measured the steady state of plasma virus after infusing Langat virus intravenously in monkeys (see Fig. 2). These data have recently been reviewed by McLean et al. (2000) to illustrate the parallels with HIV dynamics. Langat virus produces a plasma viremia. In non-immune animals, the mean clearance time of the virus is 20–30 min, irrespective of the steady state viral load, or set point. The higher of the two set points in Fig. 2 was achieved by a higher inflow rate of Langat virus.

For humans, the relationship between viral load and disease was first demonstrated in a study of infants infected

Table 1
Univariate and multivariate assessment of prognostic variables

Parameter	Univariate			Multivariate		
	OR	95%CI	*P*	OR	95% CI	*P*
Viral load (per 0.25 log)	2.79	1.22–6.39	0.02	2.77	1.07–7.18	0.04
Viremia	23.75	3.69–153	0.0009	34.54	0.75–1599	0.07
R+ serostatus	0.22	0.05–0.95	0.05	0.92	0.002–446	0.98

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True for: Liver transplant (Hassan-Walker et al., 1999), renal transplant (Cope et al., 1997), bone marrow transplant (Gor et al., 1998) and AIDS (Bowen et al., 1997; Emery et al., 1999).

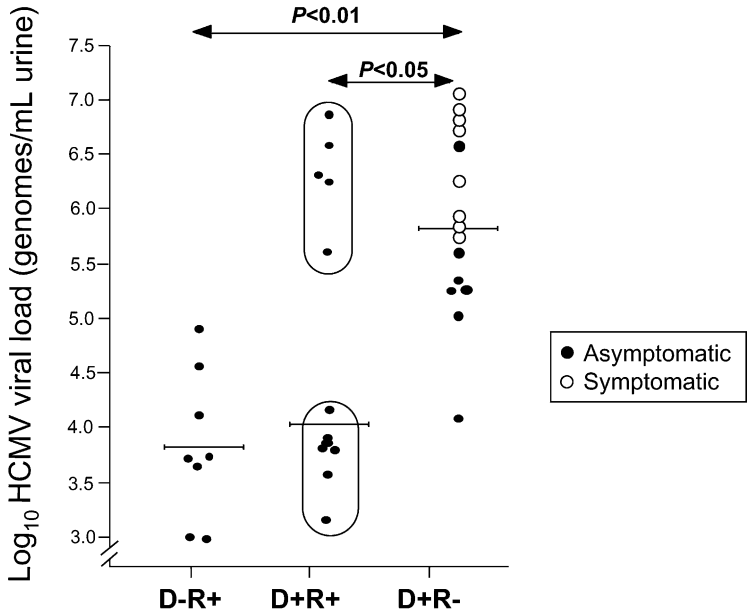

Fig. 4. Peak viral load in urine and HCMV disease in renal transplant patients. Reprinted from Cope et al. (1997) by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

with CMV infection (Stagno et al., 1975). Asymptomatic infants infected with CMV in utero excreted significantly greater quantities of virus in their urine than those infected perinatally. Symptomatic disease due to congenital CMV was associated with an additional 10-fold higher viral load in the urine than was asymptomatic infection (Fig. 3).

These data demonstrate that in systemic viral infections, such as CMV, viral load can be measured in accessible body compartments, rather than being restricted to those inaccessible areas where disease occurs (in the case of CMV, it is often the inner ear and brain). The data also imply that there is a threshold effect; moderate loads of virus may be clinically benign, but a small amount of extra virus may reach a threshold value above which disease occurs.

The pathogenic implications of viral load thresholds have been investigated in a series of studies of CMV infection and disease in patients undergoing transplantation. The correlation between peak viral load in urine and CMV disease was clear: significantly higher viral loads were associated with symptomatic disease ($P < 0.01$; Cope et al., 1997).

The donor-recipient serostatus is also a key factor in the development of CMV disease in transplant patients. If the donor is infected with the virus, but the transplant recipient is not (D+R−), there is a high risk of disease in the seronegative recipient with an attendant high viral load (Fig. 4). However, if the recipient is CMV-seropositive and reactivates their own CMV, there is a low risk of disease and, if infection occurs, the viral load is low.

The data from renal transplant patients have been analyzed by both univariate and multivariate statistical models (Table 1). Using a univariate analysis, CMV disease was linked to high viral load with an odds ratio (OR) of 2.79

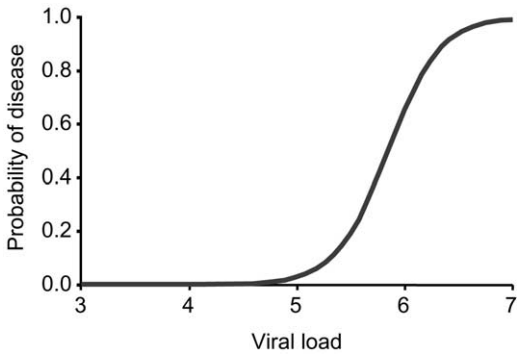

Fig. 5. The threshold concept. Reprinted from Cope et al. (1997) by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

($P = 0.02$). Viremia was strongly associated with CMV disease and a recipient-positive serostatus was protective from disease. In the multivariate model, controlling for viral load removed serostatus as a risk factor, but the significance of viral load remained unchanged. Thus, viral load is the determinant of disease, and the other factors are only associated with disease because of their link with a high viral load. Furthermore, disease occurs when viral load crosses a threshold value (Fig. 5).

Similar associations between CMV viral load and disease have also been observed in liver transplant recipients (Hassan-Walker et al., 1999), bone marrow transplant (BMT) recipients (Gor et al., 1998) and individuals infected with HIV (Bowen et al., 1997; Emery et al., 1999). The therapeutic implications of these data for patients with CMV infection are clear: antiviral drugs that prevent the viral load from reaching its threshold could reduce the bur-

den of CMV disease without necessarily eliminating active CMV replication.

3. Transmission between individuals

Factors potentially influencing viral transmission include:

- Exposure to the infectious agent from symptomatic or asymptomatic subjects
- Inoculum size
- Site of exposure
- Virus type
- Existence of more virulent viral strains
- Viral 'fitness'
- Physiochemical barriers in the recipient (pH, stratum corneum, mucus, etc.)
- Innate, heterologous or homologous immunity in both donor and recipient
- Susceptibility to, or the frequency of occurrence of, triggers to reactivation
- Genetic factors → recipient cell susceptibility or immunity

For a virus to be successfully propagated, it needs to survive and adapt to host defenses. This can be achieved by rapid replication, high viral loads, acute infections and disease, leading to solid immunity after infection (see Fig. 1, panel A). Alternatively, the virus can establish chronic, high viral load infections which evade immune responses (panel F). Herpesviruses (panels B–D) take a more subtle approach, with low viral loads and a low incidence of disease, relying on persisting/chronic infections to transmit to others.

The principles of viral pathogenesis illustrated in Fig. 1 may also be relevant for transmission and studies should explore this. For example, for HSV, the number of days of viral shedding may be a better predictor of transmission than the peak quantity of virus shed. Furthermore, the plot of days shedding versus risk of transmission might also demonstrate a threshold relationship because epidemiological data imply that most people who are exposed to HSV sexually do not acquire HSV infection (Mertz et al., 1988). This raises the question of how far to the left the threshold curve needs to be shifted (using the surrogate markers of HSV transmission) to ensure that susceptible individuals benefit from dramatically reduced risk of HSV acquisition.

3.1. Routes of transmission

In many cases the route of transmission is obvious. Rotaviruses, for example, are transmitted when a susceptible person comes into contact with infected feces, and influenza is transmitted when an uninfected individual is exposed to virus-bearing droplets produced by the coughs or sneezes from an infected person.

Several viruses are transmitted through contact with infected blood and their infectivity is a function of the amount of virus present in the inoculum. Taking needlestick injuries as an example, the risk of transmission of hepatitis B virus is higher than the risk of hepatitis C virus (HCV), which in turn is higher than the risk of HIV infection. This is directly related to viral load, and more specifically the number of viral genomes present in the amount of blood (1 μL) typically transferred in a needlestick injury (Table 2).

Table 2
Needlestick transmission

Virus	Risk (%)	Viral load (genomes/mL)	Viral load (genomes/μL)
Hepatitis B	30	10^8	10^5
Hepatitis C	3	10^6	10^3
HIV	0.3	10^4	10^1

In a study of perinatal HCV infection, women with higher levels of HCV plasma RNA were more likely to transmit virus to their newborns than those women with lower RNA levels (Thomas et al., 1998). In 155 women infected with both HIV-1 and HCV but with little or no detectable HCV RNA, the risk of perinatal transmission of HCV was low, with less than 10% of the infants becoming infected. The median concentration of plasma HCV RNA was higher among the 13 mothers with HCV-infected infants (2.0×10^6 copies/mL) than among the 142 mothers with HCV-negative infants (3.5×10^5 copies/mL; $P < 0.001$). There were no instances of HCV transmission from the 40 mothers with HCV RNA concentrations lower than10^5 copies/mL, again suggesting that a threshold effect may relate transmission risk to viral load.

The association between high viral loads and increased transmission was also observed in a study of HIV transmission among heterosexual couples discordant for HIV-1 in Rakai, Uganda (Quinn et al., 2000). The viral load was 0.5 log higher in those individuals transmitting HIV compared with those who did not transmit the virus to their partner, again consistent with a threshold effect.

The risk of transmission of HIV also increases with the number of exposures to that virus. In a study by Gray et al. (2001), the risk of transmission increased with time and correlated with younger age (Fig. 6). This suggests that there may be a qualitative element facilitating the transmission of HIV.

Other factors influencing the rates of viral transmission include:

- Circumcision (protective against HIV infection)
- Eczema (likely to increase transmission risk) or genital ulceration
- Rectal mucosa is more susceptible to HIV infection than vaginal epithelium
- HIV may predispose an individual to acquire HSV at a lower inoculum
- Pregnancy
- Genetic susceptibility of the host
- Virulence of the viral strain

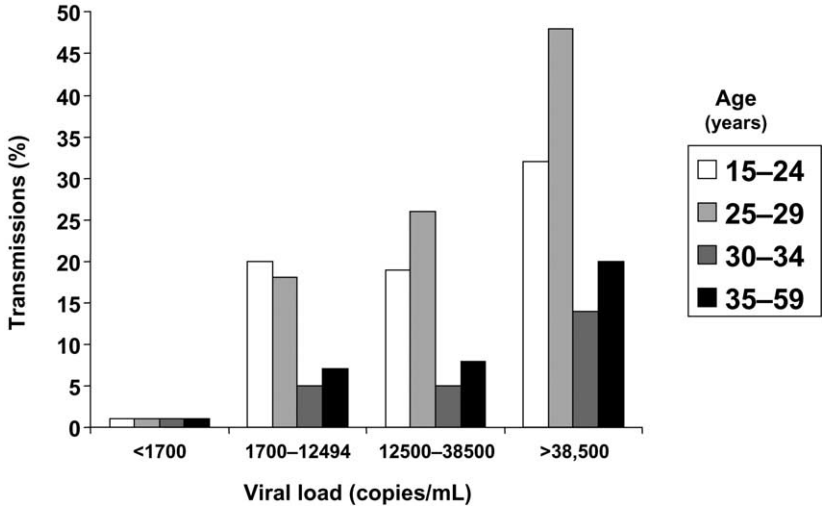

Fig. 6. Probability of HIV-1 transmission per 10 000 sex acts. Reprinted with permission from Elsevier (Gray et al., 2001).

- Gender, where the relative risk of male to female transmission of HSV is higher (Sacks et al., 2004)

3.2. Assays to detect viruses

Laboratory tests available for detecting and differentiating viral infections include:

- Amplification of target
  - cell culture
  - PCR
- Amplification of signal
  - Branched DNA (bDNA)

When selecting a laboratory test to detect evidence of infection, it should be capable of detecting all strains of the virus equally. Detection of infectious versus non-infectious virus should also be considered. Realistic limits of detection should be considered, with a goal of high sensitivity but also the need to avoid detecting latent DNA.

When blood is used as a source for serial tests, viral load is stable in the short term, so providing the set point for viruses such as HIV. However, some viruses such as CMV can be susceptible to dramatic changes in viral load in the blood and the same is true of HCV. Using urine as a source for testing has the advantage that the patient self-collects samples at appropriate time points. However, the fact that urea inhibits the PCR test should be considered when the sample is analyzed, and dilution effects due to variable fluid intake must be considered. Skin swabs are a much more variable test specimen. The swab can sample vesicles, ulcers, or intact cells. Both the randomness and the total area sampled are variables. The vigor of collection can alter the amount of cell rupture and release of DNA. Variability in the release of the cells from the swab can also occur. Finally, dilution of the sample and DNAses released from cells can both hinder viral detection. This raises the question of whether skin swabs are sufficient and accurate enough to provide the standardized biological material needed to define a parameter of transmission for HSV, or whether more sophisticated methods are required.

4. What is a surrogate marker?

A surrogate marker is a measurable parameter that correlates with a clinical endpoint, but is not necessarily directly responsible for that endpoint. For example, the measurement of HIV load in the plasma of an individual is a good marker for transmission of HIV between individuals during sexual contacts, although the HIV virus transmitted is actually present in the semen. The surrogate may also be relevant for more than one transmission route; plasma HIV is equally predictive for transmission among intravenous drug users and perinatal routes of transmission. The surrogate may also correlate with transmission in the future, or in the past; and has potential uses for diagnosis, in monitoring progression of disease and as an endpoint when no clinical parameters are measured.

Definitions of surrogate markers have been formalized by DeGruttola et al. (2001):

- *Biomarker*: characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention;
- *Clinical endpoint* (T): characteristic that reflects how a patient feels or functions or how long a patient survives;
- *Surrogate endpoint* (S): biomarker intended to substitute for a clinical endpoint in a study evaluating the impact of an intervention (I).

The goal for using surrogate markers is to provide a reliable inference about the impact of an intervention on a clinical endpoint when only the impact of intervention on surrogate can be observed. A clinical endpoint or true endpoint reflects the patient's clinical status, whereas the surrogate endpoint can substitute for that endpoint (and is not simply a correlate of the clinical endpoint).

There are many complexities and issues surrounding the use of surrogate markers, and the context in which they are used. Surrogate endpoints are widely accepted and used in Phase I and Phase II trials; however, there can be objections to their use in Phase III trials from both investigators and regulatory authorities if the surrogate is not validated. Furthermore, the validation of a surrogate inherently requires prediction or extrapolation from existing data and causal mechanisms cannot be addressed statistically or empirically. A surrogate cannot be validated even if the clinical endpoints are defined prospectively and the study then measures both in a way that prevents bias and gives a statistically significant result because the use of a surrogate inherently implies its use in some new intervention. The assumption that is non-statistical in nature is that the mechanism of action for the 'new' intervention is similar enough to those used in the statistical validation of the surrogate that the relationship estimated between the change in the surrogate and the change in the clinical response in the validation data set applies to the 'new' intervention. The FDA are asking for new validations of the newer fusion inhibitors because the mechanism of action is so different from the older generation drugs and therefore changes in viral load induced by the fusion inhibitors may not be predictive of clinical benefit in the same way as changes in viral load induced by reverse transcriptase inhibitors, for example. Such assumptions of generalizability are qualitative in nature and difficult to elucidate. This point also makes it clear that surrogates cannot be validated generally but only with respect to specific interventions that are judged to be 'similar' to those used in the statistical analyses relating the change in the surrogate to clinical changes. The definition of the scope of the problem is also important. In some settings surrogates have yielded unintended consequences of the intervention caused by completely different disease pathways.

Although the quantity of virus present is clearly important in transmission of some viruses, it is not clear whether this is the case for HSV transmission. Ideally, a surrogate marker needs to be able to identify individuals with detectable amounts of virus, and differentiate them from individuals with detectable amounts of virus which are transmissible. With HSV, the measurement of viral shedding (by sampling and PCR assay) is a surrogate for the presence of infectious virus, and it is clear that without infectious virus there cannot be transmission. Uncertainty remains about whether the inherent variability of sampling viral shedding limits its use as a validated surrogate marker.

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