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Review

Lessons from HIV and hepatitis viruses

S.L. Sacks[a], P.D. Griffiths[b], L. Corey[c], C. Cohen[d], A. Cunningham[e], G.M. Dusheiko[f], S. Self[c], S. Spruance[g], L.R. Stanberry[h], A. Wald[i], R.J. Whitley[j,*]

[a] *Viridae, Vancouver, Canada*
[b] *Department of Virology, Royal Free and University College Medical School, London, UK*
[c] *Fred Hutchinson Cancer Research Center, Seattle, WA, USA*
[d] *Harvard Medical School, Boston, MA, USA*
[e] *Westmead Millennium Institute, Westmead, NSW, Australia*
[f] *Royal Free Hospital, Academy Department of Medicine, London, UK*
[g] *University of Utah School of Medicine, Salt Lake City, UT, USA*
[h] *Department of Pediatrics and the Sealy Center for Vaccine Development, The University of Texas Medical Branch, Galveston, TX, USA*
[i] *University of Washington, Seattle, WA, USA*
[j] *The University of Alabama at Birmingham, Children's Harbor, 1600 Seventh Avenue South, Birmingham, AL, USA*

Abstract

Surrogate markers are an important component in the process of investigating management and prevention strategies, and for increasing understanding of viral diseases. The importance of surrogate markers and applied statistical models is particularly true for HIV. For HIV infection, the development of such methods provides new approaches for evaluation of HIV therapies and vaccines, and for the study of HIV transmission and its pathogenesis. The complex natural history of hepatitis B infection demonstrates that viral load is not the only predictor of transmission of this virus; for hepatitis C infection, viral load *per se* is not a prognostic factor for disease progression, but cumulative viral load may affect the outcome, and therapy is aimed at eliminating active viral replication.

Keywords: HSV-2; Surrogate; Transmission

1. Introduction

Innovative statistical methods are expected to continue to play an important role in the investigation of management and prevention strategies, and for increasing understanding of viral diseases. Such methods are needed for: (1) effective utilization of immunologic and virologic measures, (2) defining the relationship between disease markers and clinical outcomes, and (3) validating treatment decisions. Surrogate markers are an important component of this process.

The importance of surrogate markers and applied statistical models is particularly true for HIV, as the epidemic evolves and new research tools and new therapies become available. For HIV infection, the development of such methods provides new approaches for evaluation of HIV therapies and vaccines, and the study of HIV transmission and its pathogenesis. Lessons and principles from the HIV area may be useful in the study of other viral diseases, including HSV.

2. Models used in surrogacy

In the late 1980s, biostatisticians identified the problem with the use of surrogate markers of disease outcome as follows: under what condition is a test of a null hypothesis that there is no effect on a surrogate equivalent to a test of a null hypothesis that there is no effect on the true (clinical) endpoint? While important criteria were identified to test this, more realistic models are needed for 'grading' surrogates.

Freedman et al. (1992) suggested that an intervention could affect a clinical endpoint without interacting with the surrogate. They postulated that it was necessary to determine the proportion of treatment effect (PTE)—calculated by measuring the treatment effect without adjusting for the surrogate, then statistically adjusting for the surrogate and

* Corresponding author. Tel.: +1 205 934 5316; fax: +1 205 934 8559.
E-mail address: rwhitley@peds.uab.edu (R.J. Whitley).

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evaluating how the effect diminishes. The ratio of these two quantities provides the PTE; this model has been applied in a number of settings.

2.1. Models of surrogacy in HIV

2.1.1. Maternal HIV levels as a risk factor for transmission to infant

In a study of the effects of AZT versus placebo on transmission of HIV from mother to infant at delivery, Sperling et al. (1996) found that AZT reduced the overall risk of transmission by approximately 70% (22.6% versus 7.6%). The reduction of risk was accompanied by a 1.7-fold change in HIV-1 RNA concentrations in maternal plasma at delivery, suggesting that only a small part (20%) of the treatment effect was accounted for by changes in maternal plasma HIV-1 RNA concentrations. Similar discrepancies have been found in other trials of antiretroviral therapy in mother-to-infant transmission. This highlights the fact that there are limitations with using these statistical models. Variability in measurements of the surrogate is important and can attenuate the relationship of the surrogate and true endpoint. Therefore, such variability must be taken into account when assessing surrogates. It also demonstrates that variability across different studies and settings may be important, although meta-analyses of multiple studies may overcome this aspect.

The models for surrogates have been refined to follow treatment over time. In the model proposed by Daniels and Hughes (1997), there is a latent or unobserved variable that is influenced by intervention and this independently affects both the surrogate and the true endpoint. However, since the surrogate does not impact the true endpoint directly, this model allows for the possibility of measurement errors.

Multiple studies can be analyzed using a meta-analysis, where the same basic structural relationship holds for each of the studies included. Observational study data can play an important part in such models where there is a complexity of factors that affect the unobserved latent variable.

Fifteen Phase II/III randomized clinical trials in HIV were analyzed by Daniels and Hughes (1997), using the meta-model of class of antiretroviral drugs. The surrogate considered was change in CD4 count from baseline to 6 months, and the clinical endpoint was AIDS-defining events or death at 2 years. Since both the surrogate and the true endpoint were observed in these 15 trials, the treatment impact on both the surrogate and the true endpoint could be jointly estimated. The results showed that:

- Interventions that had a larger CD4 benefit were also associated with greater clinical benefit.
- The predicted clinical benefit from the observed CD4 benefit has low precision.
- Larger benefits of the interventions in terms of both CD4 count and the true clinical endpoint were seen only in placebo-controlled trials, and this may limit the ability to generalize from these data.

3. HIV—clinical issues in surrogacy

The treatment of HIV infection has been one of the success stories of recent years. In the mid-1990s, HIV was the leading cause of death among young men in the USA, but by 1997 it had fallen dramatically. This has been largely due to the prevention of opportunistic infections that accompany HIV.

3.1. Viral load and CD4 counts as surrogate markers for HIV progression

3.1.1. Viral load

The importance of HIV viral load in the development of AIDS has been well known for many years. In a study looking at the likelihood of developing AIDS over 2 years, the amount of illness was directly related to viral load regardless of the CD4 count (Mellors et al., 1997) and the data also suggested that there are thresholds for progression. While viral load is a good surrogate for the progression of HIV disease, viral load in combination with CD4 count is an even better surrogate.

3.1.2. CD4 count

As opportunistic infections occur mainly in those HIV-positive individuals with low CD4 counts, a lot of work has focused on who should be treated and on the short-term risks. In the Multicenter AIDS Cohort Study, it was found that the risk of developing illness was very low in patients with CD4 counts of >200, even when their viral loads reached 50–60 000 copies/mL (Mellors et al., 1997). These data have led to controversies about the optimal time to initiate treatment strategies, as it appears that short-term risk of illness is better predicted by CD4 count than by viral load. While viral load drives CD4 destruction, in the short term it is the current CD4 count that determines risk of illness.

As it is now possible to monitor people who are at lower risk of illness, and it is possible to postpone treatment, the question has become: should treatment be postponed? In patients whose CD4 counts remain above 100, it is not entirely clear whether treatment reduces mortality, as these patients are less likely to have opportunistic infections (MacArthur et al., 2001). In a study of more than 1200 patients initiating triple therapy, the risk of death at 30 months was much higher in those patients with lower CD4 counts compared with those having higher CD4 counts (Fig. 1) (Montaner et al., 2001).[1]

3.2. Viral load and CD4 counts as surrogate markers for opportunistic infections

The risk of opportunistic infection is fairly low in those patients with CD4 counts greater than 100, but below this level the risk increases considerably (Fig. 2).

[1] Reprinted from Montaner JSG, et al, Mortality risk stratified by CD4 count, Clin. Infect. Dis. 38 (Suppl. 2) S73–S79. Copyright © 2004, University of Chicago Press.

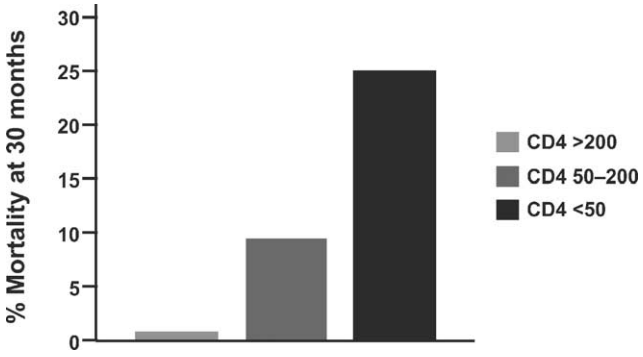

Fig. 1. Mortality risk stratified by CD4 count (Montaner et al., 2001).

CD4 count can be used as a surrogate marker to measure virologic responses to HAART. In a study measuring virologic responses in 553 patients starting HAART who were stratified by initial CD4 count (Chaisson et al., 2000), a durable response was more likely in patients with CD4 counts above 350. Therefore delaying therapy until CD4 counts fall to lower levels may result in poorer response, possibly due to toxicities or lower rates of adherence associated with those who delay care until there are lower CD4 counts. Other studies confirm that viral suppression rates are lower when treatment is initiated at lower CD4 counts compared with rates in patients with higher CD4 counts (Fig. 3; Phillips et al., 2001).

CD4 count can also be used to predict mortality. In patients with HIV participating in the Hospital Outpatient Study (HOPS), the mortality rate in patients with delayed treatment was compared with those where treatment was not delayed. The results clearly showed that the mortality rate (deaths per 1000 patient-years) was much higher in patients with delayed treatment and low CD4 counts compared with those who did not delay treatment. In contrast, there was much less difference in the mortality rate between patients with higher CD4 counts regardless of whether they delayed treatment or not (Palella et al., 2002).

4. Transmission of HIV

4.1. Horizontal transmission

Viral load was the main predictor of transmission of HIV in the recently published study in rural Uganda, which followed 415 HIV serodiscordant heterosexual couples to measure the incidence of HIV infection (Quinn et al., 2000). Overall, there were 90 cases of transmission, with no gender differences. However, age was a factor—transmission rates were lower among older people when the data were normalized for the number of sex acts. Circumcision was also protective—there were no cases of transmission to men who were circumcised. The study found no cases of transmission if the HIV-positive partner had a viral load less than 1500 copies/mL, and the risk of transmission increased by 2.45-fold per log viral load increase. Ninety-five percent of the transmission occurred in people with viral loads of >3000 copies/mL, implying that even partial suppression of viral replication would potentially prevent most of the cases of HIV transmission. This could be taken as evidence for a threshold effect as discussed in the first manuscript in this supplement, but does not rule out the possibility that there is no true threshold below which no transmission will occur.

Transmission of HIV is also more likely in the early period following initial infection. The probability of transmission is 8.2 per 1000 heterosexual coital acts in the first 6 months after infection, which then declines to 1 per 1000 coital acts, before increasing again to 5 per 1000 coital acts in the 5–15-month period before death (Wawer et al., 2003). This probability correlates clearly with viral load, and viral load is the main predictor of transmission.

4.2. Vertical transmission

In a recent study of vertical transmission, it was shown that it is the viral load at the time of delivery, rather than the CD4 count, which influences transmission (Mofenson et al.,

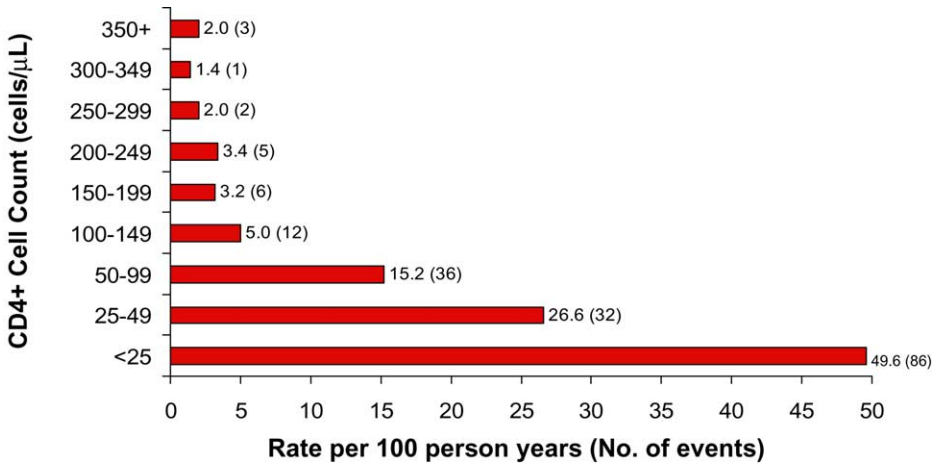

Fig. 2. Low CD4 counts correlate with increased risk of OI. Reprinted by kind permission of MacArthur et al. (2001).

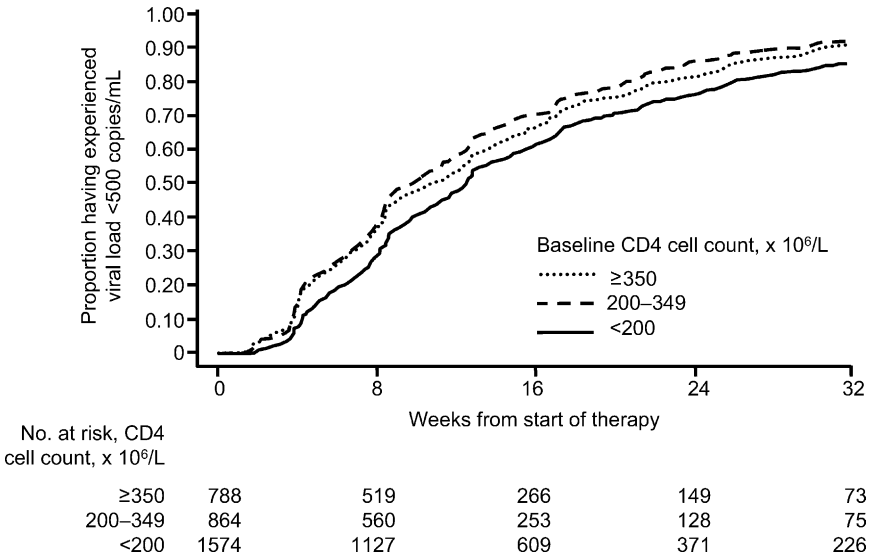

Fig. 3. Virologic response to treatment by CD4 count. Reprinted from Phillips et al. (2001) with permission from the American Medical Association.

1999). Moreover, there were no cases of transmission to the infant if maternal viral load was less than 500 copies/mL at delivery. However, it is clear that there is no viral load value below which transmission cannot occur. A review of seven European and American prospective studies identified 44 cases of vertical transmission of HIV-1 among 1202 women with a peripartum viral load of <1000 copies/mL (Ioannidis et al., 2001). The transmission rate was 9.8% for untreated women, compared with 1% for women receiving ART (OR 0.10).

Similarly, the risk of infant infection from breast-feeding is influenced by viral load in breast milk, which is highest early after delivery. For every 10-fold increase in breast milk viral load, the risk of transmission doubles ($P < 0.001$) (Rousseau et al., 2003) (Fig. 4). In a study of 275 women followed for up to 2 years after delivery, the viral load in colostrum/early milk was significantly higher than that in mature breast milk collected 14 days after delivery ($P \leq 0.004$). Those mothers who transmitted HIV-1 to their infants had both significantly higher breast milk viral RNA throughout lactation and more consistent viral shedding, compared with mothers who did not transmit HIV-1.

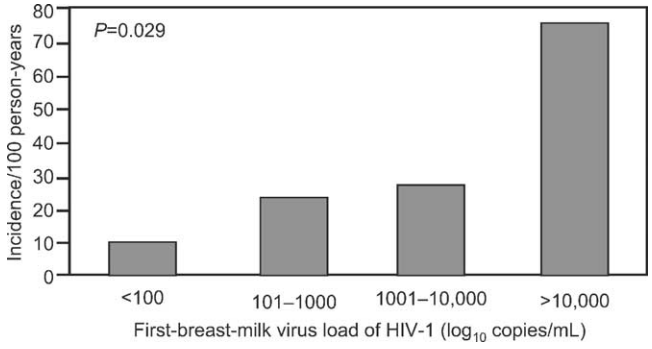

Fig. 4. Breast milk HIV viral load as a risk factor for transmission. Reprinted from Rousseau et al. (2003) with permission from the University of Chicago Press.

Chemoprophylaxis is a standard procedure to reduce the risk of transmission of HIV to the infant. Studies are also planned to prevent sexual transmission of HIV using tenofovir in the seronegative partner at risk of HIV infection. Post-exposure prophylaxis is one strategy being explored and utilized in prevention of HIV transmission.

In conclusion, within the context of HIV infection, viral load provides a tool for staging risk of illness, while the clinical impact of CD4 count is still unclear. Measurements of viral load are an excellent tool for monitoring drug effects, and provide important information about the risk of transmission.

5. Hepatitis B virus

Hepatitis B virus infection has a complex natural history (Fig. 5). Most adults who are infected with hepatitis B will have an immune response, develop hepatitis and then clear the virus. Conversely, when the infection is transmitted from a mother to an infant, immunologic tolerance develops and the disease often does not resolve, leading instead to chronic infection with cirrhosis, liver failure or hepatocellular carcinoma in around 30–40% of people. However, viral load is not the only predictor of transmission of hepatitis B.

Individuals who become infected at a young age are generally neonates who are HBe antigen-positive ($HBeAg^+$) and exposed to high levels of DNA (10^8–10^{12} and higher). Liver histopathology and liver function tests remain normal for long periods of time, demonstrating that viral load does not predict disease in these individuals. Some individuals spontaneously seroconvert to anti-HBe; this can be used as a marker of a reduction in virus levels, as these patients simultaneously lose HBeAg, seroconvert to anti-HBe and

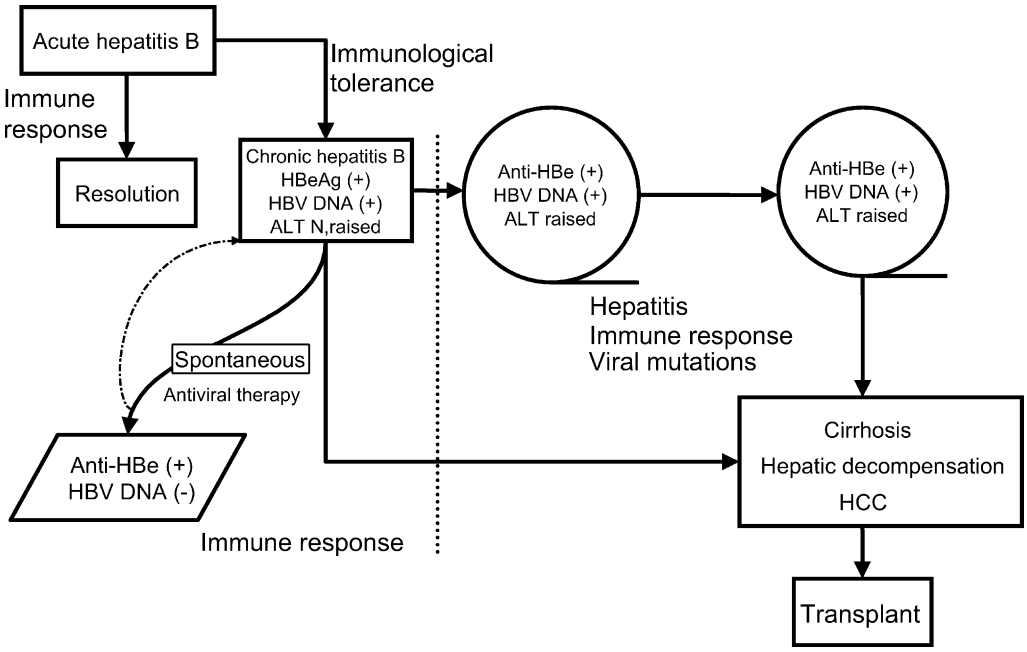

Fig. 5. Natural history of hepatitis B infection.

become negative for hepatitis B DNA ($<10^5$ genomes/mL). The disease is benign in this state, but the outcome is very variable. Patients may revert to higher levels of HBV replication, or fluctuate between higher and lower levels of viral replication with attendant liver injury accompanying these alterations. Replicating virus (usually identified by the presence of HBeAg) determines the outcome of disease. Those individuals who remain HBeAg-positive have a greater risk of developing hepatocellular carcinoma than those individuals remaining HBeAg-negative (Fig. 6).

Prognostic factors for progression to liver cirrhosis include older age, persistence of HBV DNA, virus genotype C in Asian patients, recurrent acute flares, histologic stage, alcohol consumption and coinfection with HIV, HCV or HDV (Table 1).

Earlier studies also suggest that survival is associated with clearance of HBeAg. This clearance can occur spontaneously, or be induced by interferon treatment. The continuation of active hepatitis B viral DNA replication is associated with decreased survival (de Jongh et al., 1992; Realdi et al., 1994). In individuals who remain positive for hepatitis B DNA, the risk of hepatic decompensation, liver failure, complications and clinical sequelae of liver disease or liver-related death is increased, thus providing a rationale for antiviral therapy.

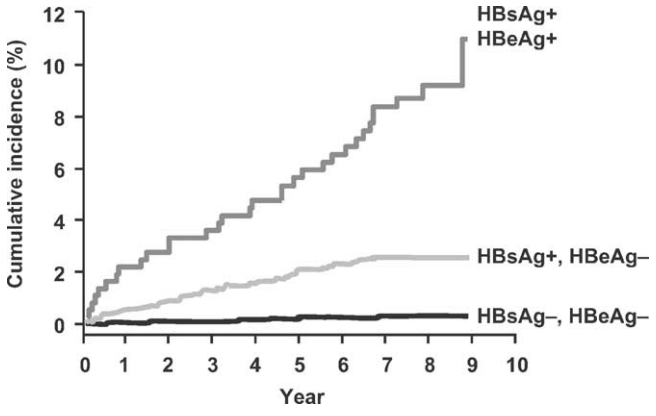

Fig. 6. Risk of developing hepatocellular carcinoma by antigen status.

5.1. Antiviral therapy in hepatitis B virus infection

Reduction in viral loads by antiviral therapy is associated with improvement in hepatic histology. For example, in recent studies with adefovir liver biopsies taken before and 48 weeks after adefovir dipivoxil treatment in 515 patients with chronic hepatitis B infection showed that the agent significantly improved both necro-inflammatory and fibrosis scores, compared with placebo. (Marcellin et al., 2003) (Fig. 7). Improvement in serum aminotransferases and a consequent decrease in ALT levels are seen when viral replication is inhibited. Histologic improvement is also associated with decreases in ALT levels. However, the interrelationships between seroconversion, HBeAg loss, low viral DNA load and therapy are as yet unclear, and the significance of these markers in the natural history of the disease is not known.

5.2. Transmission of HBV

5.2.1. Vertical transmission

Many countries carry out routine immunization of infants for hepatitis B and it is clear that vaccination successfully

Table 1
Prognostic factors for progression to cirrhosis in hepatitis B infection

Factor	*P*-value	Reference
Older age	0.0001	Liaw et al., 1988; Fattovich et al., 1991; Brunetto et al., 2002
HBV, DNA persistence	0.0001	Fattovich et al., 1991
Virus genotype C	0.001	Kao et al., 2000
Recurrent acute flares	0.001	Liaw et al., 1988
Histologic staging	0.0002	Liaw et al., 1988; Fattovich et al., 1991
Alcohol consumption	0.001	Ikeda et al., 1998
HCV, HDV coinfection	0.001	Fattovich et al., 1987; Roudot-Thoraval et al., 1997.
HIV coinfection	0.02	Colin et al., 1999

interrupts vertical transmission. However, vaccine failures can occur, which may be influenced by factors such as gestational age, HBV variants or high viral loads in the mother; breast-feeding, however, is not a factor. Prophylaxis with antiviral agents may therefore theoretically prevent transmission. One failure has been reported in a mother receiving lamivudine in the third trimester (Kazim et al., 2002) and further trials are in progress.

5.2.2. Liver transplant recipients

Outcome in liver transplant recipients who become reinfected with hepatitis B is frequently poor. Lamivudine prophylaxis is effective in this setting, although potentially it may be limited by the development of resistance. Combination therapy with lamivudine and immunoglobulin or adefovir dipivoxil reduces reinfection rates to 0–5%. Reactivation of hepatitis B in recipients of anti-HBc positive livers may also occur.

5.2.3. Healthcare workers

In a cross-sectional study of transmission of hepatitis B by healthcare workers, it was possible to define a level of virus above which transmission of hepatitis B during conduct of exposure-prone procedures could not be excluded. The lowest HBV DNA level in a transmitting surgeon was found to be 4×10^4 copies/mL (Corden et al., 2003). Many carriers had HBV DNA viral loads below the level usually nominated as the threshold to begin antiviral therapy. It is plausible that reducing the viral load of hepatitis B with chain terminating antiviral therapy will inhibit needlestick transmission of HBV.

6. Hepatitis C virus

Hepatitis C virus infection is a global problem. The factors affecting progression to severe liver disease are only partly elucidated, as attention has been focused primarily on characterizing the factors that facilitate the disease course and their impact on antiviral therapy. Viral load per se is not a prognostic factor for disease progression, but cumulative viral load may affect the outcome. Therapy is aimed at eliminating active viral replication, with the benefits of inhibition of fibrosis progression, prevention of hepatic failure, and prevention of hepatocellular carcinoma.

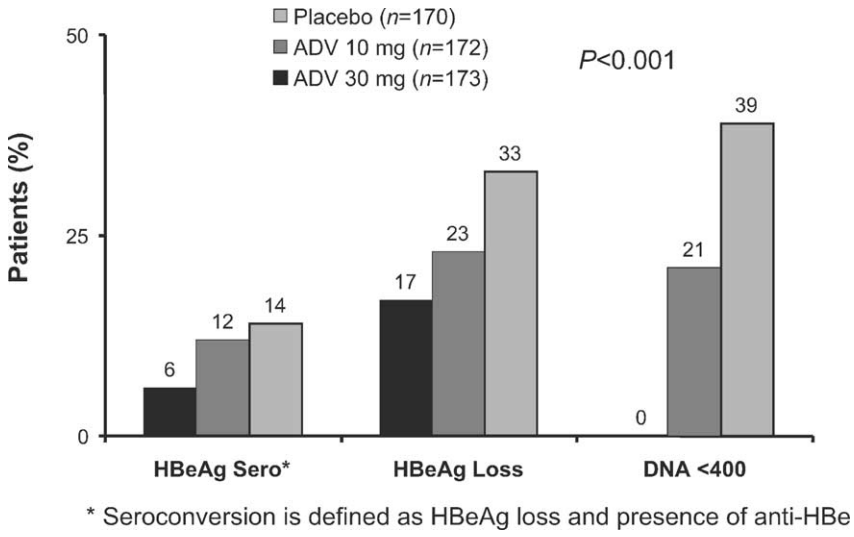

Fig. 7. Serologic/virologic change after 48 weeks of adefovir dipivoxil treatment (Marcellin et al., 2003).

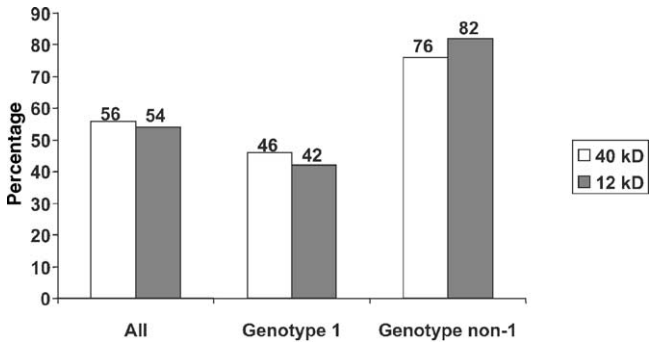

Fig. 8. Virologic response to pegylated interferons. Reprinted from Zeuzem et al. (2001) with permission from the American Gastroenterological Association.

Viral dynamics play an important role in treatment responses; for example, the degradation rate of infected cells is HCV-genotype-dependent (Zeuzem et al., 2001). The response to treatment with pegylated interferons is greater for individuals infected with non-type-1 HCV (Fig. 8; Zeuzem et al., 2001).

While the data are limited, it appears that individuals who have sustained virological response are most likely to show improvements in hepatic histology. It is uncertain whether suppression of viremia, will result in long term histological improvement. Such studies are in progress.

6.1. Viral kinetics of hepatitis C virus

Hepatitis C viral kinetics have been widely investigated. In the first phase, there is a sharp decay in viral RNA within 24 h of treatment, which may be a result of the inhibition by alpha-interferon of viral production. In the second phase, there is a more gradual and variable decline which may be due to the rate of killing of infected cells (δ). The second phase is thought to be a good predictor of sustained viral response. However, many assumptions have to be made about the mechanism of the second phase of viral decay, including the role of cell death. The mechanism of action of interferons is complex, involving many genes, and is therefore difficult to extrapolate. The effect of using pegylated interferons or ribavirin needs to be examined in greater detail. Several other confounding variables exist, such as body weight, viral genotype and degree of fibrosis.

Studies have demonstrated that virological responses in the early phase of therapy can be used to predict the results of that therapy. A recent study has suggested that early viral kinetics may predict response. A retrospective analysis of two studies indicated a strong link between the degree of viral load reduction during the first phase, and the subsequent second phase decline slope (Layden et al., 2002). There are strong correlations for both the effectiveness of interferon and the viral load at the end of the first phase, and viral load with the rate of second phase slope ($P < 0.001$).

Despite improvements in treatments, a considerable proportion of patients do not obtain a sustained virological response. Fifty-five to sixty percent of patients with genotype I virus do not clear virus. Sustained virological responses are observed in 80% of patients with genotypes 2 and 3. The early assessment of response to antiviral treatment is of key importance in providing a rationale for stopping antiviral therapy and thereby preventing unnecessary adverse events, or unsuccessful treatment. It has been suggested that early changes in HCV RNA levels during treatment with pegylated interferons or ribavirin may accurately predict eventual response; those patients who do not have a 2-log drop at 12 weeks, or become PCR-negative, have almost no chance of a sustained virologic response (the negative predictability is 98%).

To conclude, for both hepatitis B and C infection the goal of therapy should be a profound and durable viral suppression as defined by sensitive assays. Clearance of HBV DNA is difficult, and long-term antiviral therapy will be needed to predict meaningful reductions in DNA levels. Additional prospective studies are needed to determine the desirable level of viremia for both viruses.

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