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Problems in diagnosing viral hepatitis

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Abstract

The most reliable method of making a specific aetiological diagnosis of chronic viral hepatitis would be to identify virus specific cytotoxic T lymphocytes responsible for the killing of virus infected hepatocytes in each patient's liver. Unfortunately, this can not be proposed for routine diagnosis and surrogate tests are required. The detection of virus markers, and even of the virus itself, does not imply that liver damage is caused by virus infection. Indirect markers of the host's antiviral immunoresponse have to be used to confirm more specifically the diagnosis of viral hepatitis. IgM antibodies against viral antigens implicated in the elimination of the virus seem to be suitable alternative candidates. Significant changes in the serum values of viraemia and aminotransferases occur within a few days, while a significant variation in liver histology takes much longer. Only the kinetics of the highly variable parameters can be used for an appropriate study of the relationship between viraemia, antiviral immunoresponse, and liver cell necrosis. Quantitative and dynamic analyses of hepatitis virus markers seem the most suitable and reliable methods of monitoring the patients eligible for antiviral treatment and identifying the most appropriate time to start this.

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Molecular hybridisation techniques exploiting the natural properties of nucleic acids and enzymes which regulate DNA synthesis and replication have become a major research tool. They have been used to identify new genes and viruses and to unravel the mysteries of genetic heterogeneity. These techniques have contributed with innovative strength to speed the course of knowledge in the pathobiology of virus infection and liver disease, and are now being introduced into the diagnostic laboratory.

Before the polymerase chain reaction (PCR) revolution, viruses were detected by isolation and growth in tissue cultures or cell lines, or by molecular hybridisation using nucleic acid probes. The sensitivity of these techniques was limited to hundreds of thousands of virions.[1 2] Now PCR can detect a few viral genomes, amplifying a target nucleotide sequence.[3] Therefore, we have to consider viruses as bacteria which may be undetectable in biological specimens, but can be amplified in culture (Table I). This leads to the detection of viruses in individuals who are negative by detection of conventional viral markers, and thus to a change in diagnostic criteria. With analogy to bacteriology, it is well known that 'sterile' does not mean the absence of bacteria, merely presence in a number of defined cut-off value. Now that viruses can be detected with absolute sensitivity after PCR amplification, diagnostic and therapeutic decisions in virology should rely on quantitative cut offs (Table I).

TABLE I *Common diagnostic criteria in bacteriology and virology*

Bacteria	*Viruses*
Amplification by culture	Amplification by PCR
Diagnostic quantitative cut off between 'sterile' and infection	Cut off???

This paper analyses some of the current diagnostic problems and discusses possible means of overcoming them.

Quantitative detection of viral markers

Many valuable reviews describe in detail the nucleic acid amplification techniques and discuss their advantages and the problems associated with them.[3 4] A major advantage is their absolute sensitivity, while a major problem is the detection of false-positive results caused by contamination of the sample with amplification products. False-negative results are also possible, because of genetic heterogeneity at the site of the oligonucleotide primers' hybridisation. This is a minor problem, however, if highly conserved genomic regions are selected as targets.

The improvement in sensitivity leading to the detection of minute amounts of virus in unexpected circumstances and individuals who are negative for conventional virus markers, has raised medical scepticism and has overemphasised the risk of false-positive results. However, many of the controversial results have been confirmed to be specific by the introduction of contamination control systems, such as PCR carry-over prevention methods.[5 6]

A major need for the application of PCR to diagnostics is to make it suitable for quantitative analysis. The efficiency with which oligonucleotide primers hybridise all appropriate target sequences at each cycle of amplification depends on the number of oligonucleotides which rehybridise to themselves, and on the quantity of enzymes and dNTPs which are needed for polymerisation. Most of the quantitation problems derive from the variability of these factors. Terminal dilutions of each biological

sample are currently used for quantitative analysis, but they are costly and unpractical.

One possibility is the use in each assay of a dilution curve of measured amounts of an unusually sized target nucleic acid (including an insert that makes it distinguishable from the typical target sequence because of a different migration length in gel electrophoresis).[7] Another possibility is the quantitation of the PCR product by immunometric assays, for instance using an enzyme labelled monoclonal antibody that recognises specifically the stechiometric bonds of double stranded DNA hybrids.[8] Even if a precise quantitative analysis is difficult to obtain, however, for simple clinical aims such as the evaluation of the response of viraemia to treatment, we can obtain valuable data by semiquantitative, single step PCR techniques.[9]

Quantitative nucleic acid amplification assays will not solve the problem of a specific aetiological diagnosis of chronic viral hepatitis. In fact, the detection of a consistent number of viruses in a patient with liver disease does not imply that the liver damage is caused by the virus. For instance, in hepatitis B virus (HBV) infection, florid virus replication can persist for years without liver damage if the host's immune system does not react against viral antigens.[9 10] Liver disease begins as soon as immunotolerance is lost and the virus infected cells start to be eliminated, therefore hepatitis B represents an injurious way of recovering from the infection. In addition, many patients have multiple causes of liver damage. For example, a chronic carrier of HBV may also have Wilson's disease and, therefore, the hepatologist needs to rely on specific markers of virus induced liver damage rather than on markers of virus infection and replication alone (Table II).

In HBV infection diagnosis, the improved sensitivity of new IgM anti-hepatitis B core (HBc) tests and the use of new assay formats which do not allow serum IgM values to influence the results of the assay, have changed the diagnostic capabilities of this assay.[11-14] Serum IgM anti-HBc, detected with absolute sensitivity (10 IU) is found in any form of liver disease caused by HBV, independent of the duration of virus infection. Raised serum anti-HBc IgM values (higher than 600 IU) are usually detected after an acute flare up of alanine aminotransferase (ALT) activities (two to four weeks) which occurs in primary hepatitis B after recent HBV infection, but also in acutely relapsing chronic hepatitis B.[11 12 15 16] Therefore, only the characterisation of the sedimentation rate of IgM anti-HBc can help to distinguish primary from relapsing acute hepatitis B, as 19S IgM prevails in the former and 7S IgM is detected in the latter. Using automated assays it is now possible to quantitate serum IgM anti-HBc and measure as low as 10 IU (PEI; Paul Erhlich Institute) of this antibody.[11-16] This allows the identification of patients with either acute or chronic HBV induced liver disease, distinguishing them from hepatitis B surface antigen (HBsAg) carriers with liver damage unrelated to HBV. All sera from HBsAg negative controls and HBsAg carriers without liver disease, or patients with chronic hepatitis D, have lower than 10 IU of IgM anti-HBc.

TABLE II *Aetiological diagnosis of viral hepatitis (acute or chronic)*

	HBV	*HCV*	*HDV*
Serological markers of:			
Infection	HBsAg	anti-HCV	anti-HD
Replication	HBV-DNA	HCV-RNA	HDV-RNA
Disease	IgM anti-HBc	IgM anti-HCV?	IgM anti-HD

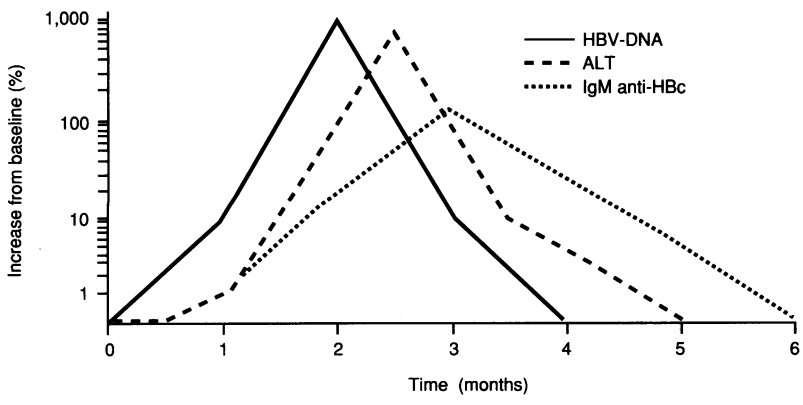

Figure Kinetics and temporal relations between the maximal peak values of alanine aminotransferase (ALT) hepatitis B virus (HBV)-DNA, and IgM anti-HBc during hepatitis B exacerbation.

Kinetics of hepatitis viral markers

Viraemia and serum ALT and IgM anti-HBc values fluctuate with significant peaks in patients with chronic hepatitis, and exacerbations of asymptomatic hepatitis are frequently separated by periods of uneventful disease course. All episodes of ALT flare up are associated with an increase in IgM anti-HBc values and are preceded by an increase or reappearance of viraemia.[14]

We analysed the relation between serum values of viraemia, transaminases, and IgM anti-HBc in 52 untreated chronic hepatitis B patients followed up for one year by monthly semiquantitative assay. In 96·2% of ALT flare ups, the chronological sequence was: HBV-DNA reactivation, followed by ALT flare up, and then an increase in the value of IgM anti-HBc (Figure), suggesting that this pattern identifies the 'true' hepatitis B exacerbation. Quantitative correlations were not found between HBV-DNA, ALT peaks, and IgM anti-HBc increases, but a significant inverse relation was present between the basal viraemia value and its increase ($r=-0{\cdot}682$; $p<0{\cdot}001$). This indicates that a rapid and noticeable increase in serum HBV-DNA values triggers the cytolytic reaction.

Monthly semiquantitative determinations of HBV-DNA, ALT, and IgM anti-HBc seem to be the most suitable and reliable approach for monitoring chronic HBV infection, and can be proposed to follow up patients eligible for or undergoing antiviral treatment.[13 14 17] The same type of quantitative and dynamic criteria should also be applied to hepatitis D virus (HDV) and hepatitis C virus (HCV)

infections, as a guide for the appropriate time to start treatment. It is well known that patients with high ALT values and low viraemia at baseline respond better to treatment, but only a precise study of the kinetics of viraemia, ALT, and IgM antiviral response can help to identify these patients or select better the most suitable phase of chronic hepatitis virus infection for the start of antiviral treatment.

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