

VIRAL HEPATITIS

An Overview

While acute, infectious hepatitis has been known for centuries and formally recognized in 1885, the first infectious agent (HBV) was not identified until the 1960's. Today, viral hepatitis has become a veritable alphabet soup of etiologic agents. Viral agents with primary effects on the liver include:

- Hepatitis A
- Hepatitis B
- Hepatitis C
- Hepatitis D (delta)
- Hepatitis E
- Hepatitis F
- Hepatitis G
- Hepatitis Sen-V

Because hepatitis constitutes a generalized pattern of inflammatory injury in the liver and because of the central role of the liver in metabolism, just about all viruses are capable of involving the liver. However, there are at least eight distinct viruses, which specifically seem to infect and damage hepatocytes. No two of these originate from the same viral family! There are also many other causes of hepatitis ranging from drug reactions, chemical toxins, sepsis or shock; however the most common cause of hepatitis in the United States is a viral infection.

The diagnosis of *hepatitis* usually rests on clinical assessment: history, physical and general biochemical findings. Only serological testing can make the diagnosis of the type of viral hepatitis. The different forms of viral hepatitis have similar clinical signs and symptoms, but differ in their means of spread, infectivity and potential outcome.

About 70% of all patients with type A hepatitis, 50% of patients exposed to type B hepatitis, and 80% of patients with type C hepatitis recover without overt clinical symptomatology. These patients do not become jaundiced, nor do they complain of any acute illness. For types A and B hepatitis lasting immunity results. Investigational studies suggest that immunity does not occur following exposure to HCV.

Approximately .05-.1% of all cases of type A hepatitis and 1-3% of adult patients with acute, icteric type B hepatitis develop a *fulminant hepatitis*, which is associated with hepatic failure, signs and symptoms of hepatic encephalopathy and death. Serological hepatitis markers in fulminant hepatitis are similar to those in acute hepatitis. Bilirubin levels may increase and a prolongation of prothrombin time commonly occur.

Hepatic inflammation lasting at least 6 months is the classic definition of chronic hepatitis. While chronicity is unknown in humans infected with type A or E hepatitis, it occurs in 5-10% of all cases of type B hepatitis and in the majority of cases of type C hepatitis.

Epidemiology of viral hepatitis

Hepatitis A Virus (HAV)

Hepatitis A virus (HAV) is endemic globally although there are wide variations in prevalence throughout the world. In excess of 80% of blood donors in Taiwan, Belgium and Yugoslavia have antibodies against HAV. In the United States, 35-45% of the adult population have anti-HAV markers. Type A hepatitis accounts for 20% of all viral hepatitis in the US. Type A hepatitis spreads primarily via the 'oral-fecal' route. The virus is relatively hardy and is commonly associated with poor sanitary conditions and overcrowding. The disease is common among institutionalized persons (prisons, military groups, mentally retarded), children in day care centers, male homosexuals and drug addicts. The prevalence of this disease is NOT increased among health care workers or dialysis patients. Outbreaks have been associated with contaminated food and water. Mollusks such as clams, muscles and oysters, which have been removed from contaminated waters, are a particular concern because they concentrate the virus.

Approximately a week after ingestion, HAV can be demonstrated in the liver by immunofluorescent techniques. The virus propagates in hepatocytes, where it is non-cytopathic. It is secreted in large amounts into the biliary system. During the prodromal period, intermittent viremia can occasionally be demonstrated. The appearance of antibodies directed against hepatitis A antigen usually accompanies the onset of symptoms. Fecal shedding of the virus continues for 7 - 14 days after the first appearance of antibodies.

Hepatitis B virus (HBV)

Type B hepatitis is spread mainly by the parenteral route. It is commonly associated with the transfusion of blood, blood products, needlestick accidents and contaminated needles and syringes. Infectious viral particles can be found in serum, saliva, semen, vaginal secretions and breast milk. Hepatitis B virus is NOT infectious by the oral, nasal or respiratory route. It is NOT capable of crossing intact skin or mucous membrane. The prevalence of type B hepatitis is increased among multiply transfused individuals, drug addicts, medical personnel (particularly emergency room and laboratory workers) and dialysis patients.

Unlike type A hepatitis, following infection with HBV large numbers of viral particles appear in the serum. The protein lipid coat of the virus containing the HBsAg is synthesized in the hepatocyte cytoplasm while the elements of the viral core are synthesized in the nucleus. Core particles are rarely found in the blood, but large numbers of complete and incomplete viral particles containing HBsAg are present. The presence of HBsAg alone is thus not a hallmark of potential infectivity. Additional antigens found following infection with HBV include hepatitis core antigen (HBcAg) and hepatitis Be antigen (HBeAg). The "e" antigen associated with the core particle is present only during the period of peak infectivity. When the antibody to HBeAg (anti-HBe) appears, the potential for infectivity is substantially reduced. *Key point:* The level of infectivity DOES NOT correlate with the amount of HBsAg present, but since HBeAg is never present in its absence, without further investigation one must presume an HBsAg + serology to be infective UNTIL proven otherwise.

Hepatitis D virus (HDV)

The Hepatitis D virus or *Delta virus* requires the presence of HBsAg in order to replicate. Co-infection with HDV may result in a severe form of hepatitis. Because of the obligate presence of HBsAg, Delta hepatitis can be either a self-limited acute process or a chronic process. Infection with HDV can occur as a co-infection with HBV or as a superinfection of an existing type B hepatitis. Delta hepatitis should be suspected following a sudden deterioration in a patient who is reactive for HBsAg. In the United States, Delta hepatitis is usually limited to individuals with multiple parenteral exposures such as hemophiliacs and IV drug abusers. A 1984 study of 8000 individuals in Los Angeles indicated that 44% of all drug addicts carry antibodies to HDV while

26% of all hemophiliacs have been previously infected. The usual incubation period for Delta hepatitis is 30-50 days. The primary means of spread is parenteral.

Hepatitis C Virus (HCV)

Hepatitis C virus (HCV) is a major cause of hepatitis and cirrhosis worldwide. The most efficient mode of transmission of HCV is associated with percutaneous exposures to blood, but such exposures account for less than half of reported cases. Sexual, household, and perinatal transmission also seem to occur, but the risks associated with these types of exposures are still unknown. In patients with sexual contact there have been estimates that sexual transmission occurs anywhere from 0-30% of the time, however, the majority of studies suggest transmission occurs in 5% or less. Thus, the sexual transmission of HCV virus is relatively inefficient. It may be that repeated sexual exposure increases the risk of transmission, but the data to support this conclusion is very scanty. Maternal-neonatal transmission of hepatitis C is also probably inefficient. Most babies will have passive transfer of maternal hepatitis C antibodies and most studies do show a lack of viremia as detected by polymerase chain reaction at 12-24 month follow-up. *Up to 40% of patients with chronic hepatitis C have no known mode of transmission.*

Virtually all persons with acute HCV infection seem to become chronically infected. Chronic liver disease with persistently elevated liver enzymes develops in an average of 67% of the cases, independent of the source for infection. It is estimated that 30% of chronically infected patients will become cirrhotic over a 20-30 year period with liver failure developing in 25% of cases. The extraordinarily high rate of persistent infection observed in humans and the lack of protection against rechallenge with homologous HCV strains demonstrated in experimental studies in chimpanzees, suggest that HCV fails to induce an effective neutralizing antibody response. This raises major concerns for the development of effective passive or active immunization against hepatitis C, and prevention may depend on a better understanding of the factors that facilitate the transmission of HCV infection.

At least 5 different genotypes and 20 different subtypes of hepatitis C virus are now recognized. The distribution of HCV genotypes differs geographically; HCV genotypes also differ with respect to biological characteristics such as pathogenicity, sensitivity to therapy and prognosis in chronic active hepatitis.

The entire viral genome has been sequenced. The envelope region shows considerable variation, and mutant HCV infections are being described already. There are geographic variations in the prevalence of anti-HCV, but usually about 0.5% to 1% of healthy blood donors test positive. Parenteral exposure to blood, especially by transfusion or drug abuse, remains a certain means of acquiring HCV infection. The method by which millions without parenteral risk factors acquire HCV remains uncertain. Vertical transmission and sexual and family spread occur only rarely. Body secretions are free of the virus. Acute HCV infection usually is mild, and the chronic disease is often indolent. Carriers of hepatitis B virus or alcoholics who also test positive for HCV tend to have a more serious disease. Chronic HCV infection must be distinguished from autoimmune chronic active hepatitis. The most important difference is the response to corticosteroid therapy, which is good in autoimmune hepatitis and poor in HCV-related disease. Hepatocellular carcinoma can complicate HCV-related cirrhosis, usually about 20 years after infection with HCV.

Recombinant interferon-alpha is used to treat chronic HCV disease, but selection of patients, dose, and duration of therapy are uncertain. In general, 50% of patients respond to the treatment, but 50% of these will have a relapse, with an overall response rate of 25%. HCV genotypes and pretreatment serum HCV RNA concentrations are the main and independent prognostic factors of sustained response to interferon alpha therapy in chronic hepatitis C. Liver transplantation in patients with end-stage HCV disease usually is followed by infection of the graft.

Hepatitis E Virus (HEV)

In the United States, approximately 1.4% of all blood donors show evidence of previous infection with hepatitis E, but it is a major cause of hepatitis in India, central and Southeast Asia, Africa and Mexico.

The clinical manifestations of HAV and HEV infection are practically speaking, indistinguishable. The principal differences are the longer incubation period of HEV, the predominance of cholestasis and the mortality among pregnant woman.

Hepatitis E is associated with fecal-oral transmission in unsanitary conditions associate with contaminated water. The virion is a spherical, non-enveloped particle approximately 32-34 nm in diameter. Genetically, the virus is similar to, but not identical with Caliciviruses. It is a single-stranded RNA virus. Hepatitis E is endemic in southeast Asia, India, Russia, the Mid-East, Africa, and Central America. Infections tend to be an acute, self-limiting process, except among pregnant women in the third trimester. Mortality rates among infected pregnant women is between 10 and 20%. There is *no* evidence for a chronic state.

The diagnosis of hepatitis E infection is currently a diagnosis of exclusion based upon clinical presentation, a history of recent travel to an endemic area and negative serology for hepatitis A, B and C. In the United States, there is no available commercial assay for antibodies against HEV. Seroprevalence studies suggest a prevalence of .5% in the United States.

Non-A-E Hepatitis:

Despite the multitude of viruses known to cause hepatitis, 5 - 20% of community-acquired hepatitis cases or nearly 300,000 cases of acute and chronic viral hepatitis cannot be attributed to any of the known viruses or with toxic, metabolic or genetic conditions. These cases are referred to as non-A-non-E hepatitis. Among these are 50 to 80% of the remaining cases of fulminant hepatitis, 15 to 30% of patients with chronic hepatitis, and 10 to 20 % of those with acute hepatitis. Evidence has also been collected suggesting an association with other potentially severe diseases such as fulminant liver failure, aplastic anemia and end-stage liver disease.

Other viral causes for hepatitis have been reported. During the past few years three distinct flaviviruses, designated GBV-A, B & C have been cloned. These have been used to develop assays for the associated antigens and antibodies and been subsequently used to study their seroprevalence in patients with non-A-nonE hepatitis. It now appears that these viruses are probably not a significant factor in the more severe forms of community acquired non-A, non-E viral hepatitis.

In July 1999 the Medical Systems Division of American Standard Companies, DiaSorin, announced the development of a polymerase chain reaction test for detecting a new virus, Sen-V. They identified the presence of this virus in blood samples of a number of patients with fulminant, chronic, and in acute forms of non-A, non-E hepatitis. Genomic and molecular analyses have subsequently established that Sen-V is a small, single-stranded virus that belongs to the super family of Transfusion Transmitted Viruses first identified in Japan in 1997 by T. Nishizawa in patients with fulminant hepatitis and chronic liver disease of unknown etiology. In 2002, investigators from Taiwan assessed the prevalence and clinical relevance of Sen-V infection in a Taiwanese population of high-risk persons, patients with liver disease, and healthy adults. They concluded that "the prevalence of [SENV] infection is significantly higher in high-risk populations and among patients with chronic hepatitis B or C than among healthy adults in Taiwan. However, persons with [SENV] infection alone or those coinfecting with [SENV] and HBV or HCV do not have increased evidence of liver disease and risk of HCC.¹

¹ J Kao and others. Prevalence and Implication of a Newly Identified Infectious Agent (SEN Virus) in Taiwan. *The Journal of Infectious Diseases*. 2002; 185:389-392.

Other Viral Causes of Hepatitis:

Other known causes of hepatitis include the two herpes viruses, **Epstein-Barr virus (EBV)** and **cytomegalovirus (CMV)**, both of which can be transmitted by transfusion and which cause hepatitis as part of a more generalized illness.

A significant number of sporadic cases of acute liver failure (ALF) still remain of uncertain cause. The application of sensitive molecular biology techniques indicates that a few cases may be due to cryptic infections with one or more known hepatotropic agents, such as HBV and HCV. Evidence however, continues to accumulate to incriminate at least one potentially novel and transmissible agent (candidate hepatitis F). In ALF of unknown pathogenesis, survival without transplantation remains less than 20% despite recent improvements in medical management

The Structure and Replication of the Hepatitis Viruses

Type A Hepatitis:

Type A hepatitis is a member of the *picornavirus* family, which also includes the coxsackieviruses, the rhinoviruses, and poliovirus. Simple and non-enveloped, the virus is approximately 27 nm in size, consisting of a single stranded RNA molecule of approximately 8100 nucleotides surrounded by a nucleocapsid consisting of 32 capsomeres. Each capsid consists of 4 polypeptides: VP1, VP2, VP3, and VP4. The 3' end of the RNA is polyadenylated. The 5' end is terminated by a small protein known as VPg (viral protein, genomic), which is thought to enhance viral attachment to cytoplasmic ribosomes. Similar in many respects to poliovirus, the RNA contains a single large open-reading frame from which a single polyprotein is synthesized. All known viral proteins are derived from this polyprotein by post-translational cleavages catalyzed by viral proteases.

Type B hepatitis:

The virus responsible for type B hepatitis is a complex, 42 nm DNA virus of a unique virus family, the *hepadna* viruses. The intact virus is double shelled and referred to as the Dane particle. It consists of an outer surface component, which contains hepatitis B surface antigen (HBsAg) and an inner compartment containing the hepatitis B core antigen (HBcAg). Inside this core compartment is the viral genome, a short (3200 base pairs) single molecule of circular, "nicked" double-stranded DNA. The DNA has a unique structure, which is characteristic of the hepadna viruses, i.e. one of the strands of the DNA is incomplete which results in a single stranded gap region comprising between 10 and 50% of the total length of the molecule. All of the genetic information necessary to produce both HBsAg and HBcAg is found on the long, "nicked" strand. In addition to the DNA, the virus core also contains a DNA-dependent, DNA polymerase. It is thought that the gap region promotes integration of the viral DNA into the host liver cell DNA. Patients that are chronically infected with HBV may have as many as 10 complete DNA viral genomes integrated into their own liver DNA. One of the well-known sequelae of chronic type B hepatitis is hepatocellular carcinoma. The integration of the virus into the liver cell may be related to the development of liver cell cancer.

Nucleotide sequencing of the HBV DNA has demonstrated four open reading frames which are labeled "S," "C," "P," and "X". The S gene encodes for HBsAg. Curiously, there are two other start codons in front of the S gene. These two gene regions are called pre-S1 and pre-S2. Depending on where synthesis of HBsAg begins from (pre-S1, pre-S2 or S) six different size antigens can be synthesized (i.e. the three different forms can exist in either a glycosylated form as a slightly heavier protein or in a non-glycosylated state. There is some evidence that the immunologic response to the larger forms of HBsAg may be stronger than the smaller 24 kd form of the antigen. The pre-S2 region is also important because it binds to polyaggregated human serum albumin. This region of HBsAg has been referred to as the pre-SA receptor and it has been hypothesized that HBV attaches and enter hepatocytes via this receptor. The open

reading frame referred to as "C" encodes for HBcAg and HBeAg. The start codon for the C gene is located in close proximity to the nick on the long strand. It has been suggested that this may explain why HBV DNA can encode for HBsAg, but does not allow for synthesis of HBcAg or intact HBV particles. The third open reading frame of HBV DNA is "P". This gene consists of a long sequence, which overlaps three other genes. Although the protein encoded by the P gene is unknown, it is suspected that the P gene encodes a viral polymerase. The final HBV gene is "X". It is short, partially overlaps the C gene and is also interrupted by a nick on the long strand. Neither the protein encoded for, nor its function is known. The RNA of HBV is less well characterized. There appear to be two sizes (3.5 kb and 2.1 kb). The larger RNA can probably encode all the viral antigens and is a likely replicative intermediate. The smaller RNA probably encodes for HBsAg alone. HBV DNA is not synthesized by the normal semi-conservative mechanism of DNA replication; instead an asymmetric process that involves an RNA intermediate much as occurs in retroviruses synthesizes it. The DNA of the intact virus is released in the cell and the incomplete gap region on the short strand and the nick on the long strand are repaired by a DNA polymerase reaction. Transcription of the 3.5 kb RNA occurs from this repaired, supercoiled, DNA. The HBV RNA can then translate to produce viral proteins or be taken up into HBcAg particles to initiate DNA replication.

Replication of HBV DNA occurs via a reverse transcriptase, which results in a minus DNA strand being produced from the plus strand of RNA inside the core particle. The reverse positive DNA strand is then produced from the DNA (-) and the RNA is digested away. The mature partially double stranded molecule of DNA is then coated with HBsAg and exported from the hepatocyte.

Hepatitis D Virus:

Since the Delta virus surrounds its genetic material with a protective coating of HBsAg, in order to contract Delta hepatitis an ongoing infection with hepatitis B virus is obligatory. The actual viral particle consists of a small ribonucleic acid molecule and the Delta antigen.

Hepatitis Delta virus has a single-stranded RNA genome that has no homology with that of any known animal virus and a RNA genetic structure that is similar to that of plant viroids and virusoids. The hepatitis D virion envelope is made up of HBsAg and contains both HDV RNA and Hepatitis D antigen without nucleocapsid-like symmetry. In the acute phase of an infection occurring either by co-infection with HBV or by superinfection of a chronic HBV carrier, synthesis of HBV proteins is significantly inhibited.

Coinfection with HBV and the Delta virus is believed to induce a typical hepatitis which is *sometimes*, but not always, *more severe* than HBV alone. **Superinfection** with the Delta virus in an HBV carrier often leads to exacerbation of the HBV infection with the development of a severe and often fulminant disease.

Hepatitis C Virus:

Until 1988 the putative agent of non-A, non-B (NANB) hepatitis had not been found. At that point, research workers at the Chiron Corporation (California, USA) identified, by "blind expression cloning", polypeptides, which specifically bound antibodies, present in sera of NANB-patients. A fusion polypeptide (C-100) was expressed in yeast. With the C-100 antigen prototype RIA and ELISA antibody tests were developed.

It has become apparent that Hepatitis C (HCV) virus represents a variable group of agents sharing certain similarities of structure and mode of replication. The possibility that different HCV agents cause different clinical outcomes and have differing cellular tropisms is currently under investigation.

Like the flaviviruses (e.g. yellow fever virus, Dengue virus) and animal pestiviruses (hog cholera virus and bovine viral diarrhea) HCV contains positive-stranded RNA that does not

replicate through a DNA intermediate. There is evidence based upon the presence of variable and hypervariable regions in the different HCV genomes to suggest that this virus may have the ability to evade immune surveillance by rapid mutation. Genetic variations in the known human hepatitis viruses are probably the result (a) of high viral replication rates and poor or absent proofreading ability intrinsic to RNA viruses (HAV, HCV, HDV, and HEV) and (b) to a DNA virus (HBV) that uses a reverse-transcription mechanism for genomic replication. Nucleotide substitutions, deletions, duplications, insertions, and rearrangements resulting in amino acid changes may have no consequences, may impair replication, change host susceptibility, or may lead to escape from immune attack. Genetic diversity has been identified in each of the known hepatitis viruses. The importance of mutant viruses in pathogenicity, immunity, natural history, clinical outcomes, vaccine production, and responsiveness to treatment has emerged as an area for intensive study.

The hepatitis C virus contains a positive-stranded RNA genome comprising approximately 9,400 nucleotides. This sequence contains a single, large open reading frame that spans the entire genome and could encode a large polypeptide of approximately 3,010 amino acids. The sequence also contains a series of three or four highly conserved open reading frames encoding peptides of up to 28 amino acids. The virus appears to encode a large polypeptide precursor from which individual viral proteins of a structural and non-structural nature are processed post-translationally by viral-encoded proteases.

There is evidence that hepatitis C viral genome RNA sequences can be separated into at least 3 distinct groups based upon nucleotide and amino acid homologies. The molecular cloning of HCV led to the production of large quantities of viral protein from recombinant organisms. When purified the recombinant polypeptide was used to produce an ELISA. The first of these assays incorporated the C100-3 polypeptide, which was expressed in yeast. This polypeptide corresponds to the protein of the HCV-1 isolate. HCV-1 is one of the identified proteins of the HCV 1 class. Antibody to HCV-1 develops in nearly all cases of post-transfusion NANB hepatitis and is particularly associated with chronic infections and appears to be a good marker for the presence of infectious virus. In use, this assay appears to detect infections by HCV groups I and II. The degree of cross-reactivity with the rarer, more divergent group III is unclear. Groups I, II, and III viruses have been observed in Europe and Japan, whereas so far only group I viruses have been reported in the United States.

Subsequently more polypeptides (C-200, C33c, C22) of the HCV-genome were added to the test system, resulting in second-generation anti-HCV tests with increased sensitivity. For confirmation of HCV ELISA reactive samples, recombinant immunoblot (RIBA-2, Ortho; Innolia, Innogenetics) and dot immunoblot assays (Matrix, Abbott) were developed.

In Japan, 70-80% of all cases of chronic liver disease are associated with HCV infection. Hepatitis C virus is a typical RNA virus with a high mutation rate. At least six variants of HCV have been identified by their nucleotide sequences. Even HCV cDNA clones isolated from a single patient showed mutations of HCV, especially in envelope-coding regions. Thus HCV may change during the course of chronic hepatitis due to the high mutation rate of HCV itself and elimination of some clones by immune reactions or interferon therapy. These findings explain the higher rate of chronic HCV infection and indicate that production of an effective vaccine is difficult.

Laboratory Diagnosis of Viral Hepatitis

General Principles

Antibody responses to viral infections follow a rather predictable course. Viruses ranging from picorna viruses (Hepatitis A) to hepadna viruses (Hepatitis B) or lentiviruses (HIV-1 and HIV-2)

respond to the presence of viral antigens by mounting an immunologic response, which includes the generation of detectable IgM and IgG antibodies.

In general, the early immunologic response to virus- derived antigens is the appearance of specific IgM antibodies. Somewhat later in the course of the disease, IgG antibodies become prominent. This temporal pattern is a useful basis for differentiating acute from chronic infections.

The techniques for detecting serologic antibodies have undergone significant maturation in the past twenty years. Originally based upon the radioimmunoassay, diagnostics manufacturers have in the past years evolved equally sensitive, non-isotopic methods for detecting antigens and antibodies. These procedures continue to refine themselves in terms of sensitivity, specificity, linearity, and efficiency.

Principles of ELISA technology

Enzyme-linked immunoassays (ELISA) are widely employed as sensitive screening assays for a variety of infectious agents. The concept of an ELISA is simple. The resultant diagnostic product is not always quite so straightforward in its execution, performance or reliability. Many of the products in current use to detect HIV or hepatitis have been improved in sensitivity to remove potentially infected units from the blood supply. This improvement in sensitivity often exacts a price, namely a loss in the specificity of the assay.

Consider the HTLV-I assay as an example. The single most important component in the assay is the immunogenicity of the HTLV-I antigen. In the HTLV-I assay, the virus is propagated in a chronically infected T Lymphocyte cell line. The isolated virus is disrupted and inactivated with detergent and sonicated prior to its distribution onto a solid phase. Some manufacturers make use of a system that distributes the inactivated virus onto the walls of a 96 well Titertek tray, while others coat a specially prepared polystyrene bead. In the Abbott assay, an amount of a patient's serum or a control is added to a well with a diluent and allowed to incubate for a period of time. Any antibodies to HTLV-I are bound to the HTLV-I antigens that coat the bead. After aspiration of the unbound material and washing of the bead, goat antibody to human IgG conjugated with horseradish peroxidase is incubated with the bead-antigen antibody complex. Any unbound enzyme conjugate is then aspirated and the beads washed.

Next, o-Phenylenediamine (OPD) solution containing hydrogen peroxide is added to the bead and after incubation; a yellow-orange color develops in proportion to the amount of HTLV-I antibodies bound to the bead. The enzyme reaction is terminated by the addition of 1 N sulfuric acid, and the intensity of color is read using a spectrophotometer at 492 nm. Specimens with absorbance values equal to or than the Cutoff Value are considered reactive for antibodies to HTLV-I.

To screen for antigens, the bead is coated with human antibodies to the antigen of interest. For example, in order to detect HIV-1 antigen, the beads are coated with human antibody to HIV-1. But essentially the same principle of displacement of the color generating system is used to allow qualitative assessment of whether a specimen is reactive or non-reactive.

Confirmatory Testing of ELISA results

ELISA assays vary in sensitivity and specificity. First generation screening procedures are often suboptimal, but because of the importance attached to eliminating a known infectious risk from the blood supply, they often find their first use in this environment. Invariably, confirmatory testing follows the development of the basic screening procedure.

All highly sensitive immunoassay systems have a potential for nonspecific reactions assuring the specificity of a repeatable reactive is the neutralization assay. Neutralization assays are used to confirm repeatably reactive assays for HBsAg, Hepatitis C Virus (HCV) and HIV-1 antigen.

Standard laboratory procedures require the initially reactive specimen to be rerun in duplicate. If either of the duplicates is reactive the laboratory then employs a *confirmatory neutralization* procedure. This approach is designed to increase the specificity of the original screening procedure.

The principle of a neutralization assay can be described using the example of HBsAg. A specimen is incubated with a bead, which has been coated with antibody to HBsAg. If the specimen contains HBsAg it will be bound to the solid phase antibody. At this point, the bead is incubated with confirmatory antibody forming an antibody-antigen-antibody complex. Thus, when antibody to HBsAg: Peroxidase Conjugate is incubated with this mixture, most of the binding sites are already complexed, resulting in greatly reduced absorbance values when the HBsAg assay is completed.

For comparison, if negative human control serum (negative for HBsAg and anti-HBsAg) is substituted for confirmatory antibody, it will lack the specific anti-HBs sites present in the confirmatory antibody and consequently when the antibody to HBsAg:Peroxidase Conjugate is added there will be multiple antigenic sites available. The control therefore results in a higher absorbance value since more color is generated. By definition, confirmatory procedures usually require a reduction in absorbance of 50% or greater.

Temporal patterns can reduce the utility of a particular marker as a screening procedure. When a marker is present only transiently, and seroconversion occurs associated with antigen/antibody complexing, that marker has a relatively limited utility as a screening procedure. Two very obvious examples are HBsAg and HBeAg. Of course, these features are the foundation for using particular markers as the basis for staging the disease process.

Acute Type B Hepatitis:

The typical incubation period for type B hepatitis averages 75 days and ranges from 40-180 days. Important antigenic markers of hepatitis include: HBsAg, HBcAg and HBeAg. Unlike type A hepatitis, following infection with HBV large numbers of complete viral particles appear in the serum, as well as a variety of incomplete viral forms containing HBsAg. The protein lipid coat of the virus containing the HBsAg is synthesized in the hepatocyte cytoplasm while the elements of the viral core are synthesized in the nucleus. Core particles are rarely found in the blood, but large numbers of complete and incomplete viral particles containing HBsAg are present. The presence of HBsAg alone is thus not a hallmark of potential infectivity.

The first serologic marker to appear following exposure is HBsAg. It appears during the incubation period, prior to HBeAg and all reactive antibodies. HBsAg usually peaks at, or shortly after the onset of the elevated aminotransferase level. With clinical improvement, these serum enzymes decline, usually in association with a drop in HBsAg. In about 5% of the cases HBsAg disappears early. In others, HBsAg persists. Persistence is usually a function of peak titer. Since most diagnostic tests are reported in a qualitative manner, a general guideline of 6 months is utilized as a prognostic sign that chronic hepatitis has occurred.

HBeAg usually appears coincidental with, or shortly after, the appearance of HBsAg. Generally, HBeAg disappears prior to the disappearance of HBsAg. Ordinarily, the disappearance of HBeAg is associated with the emergence of an antibody directed against HBeAg (anti-HBe) and the peak of clinical symptomatology. Individuals who progress from acute type B hepatitis to a chronic carrier state do not seroconvert from HBeAg to anti-HBe. Failure to seroconvert after 10 weeks is commonly a prognostic sign for the development of a chronic carrier state.

Antibodies to HBc antigen can be either of the IgG or IgM classes. IgM antibodies against HBc appear prior to the onset of symptoms. Seroconversion to an IgG form of the antibody typically occurs in about 6 months and usually persists for life. The presence of anti-HBc (IgM) indicates recent infection with HBV. Approximately 6-18 months later, anti-HBc (IgM) falls to undetectable levels and only anti-HBc (IgG) remains. Although chronic type B hepatitis patients may continue

to produce low or moderate amounts of anti-HBc (IgM) conceptually the information is used to distinguish between acute and chronic infections with HBV.

Antibody to HBsAg does not arise during acute infection. Its presence is a sign of recovery and immunity from hepatitis B infection. Fifty percent of all cases demonstrate a window between the disappearance of HBsAg and the appearance of anti-HBs. Typically this window may last weeks or months. The anti-HBs antigen response following type B hepatitis is often weak. 5-10% of patients never produce detectable anti-HBs. High titers are usually a sign of persons who have had multiple exposures to the virus (hemophiliacs, multiply transfused individuals).

Chronic Type B hepatitis:

When **HBsAg persists for longer than 6 months**, the individual is said to have entered a phase of chronic hepatitis. Chronic hepatitis can persist from 1-20 years, it can be associated with mild symptomatology and a variable course of liver disease ranging from active to inactive or subsiding or it can take a more aggressive course resulting in significant liver impairment. The critical factor is whether the patient continues to produce DNA polymerase and HBeAg.

- Patients who have undergone HBe seroconversion (i.e. HBeAg -, anti-HBe +) tend to be older, have usually been carriers for a long time, tend to have normal aminotransferase levels, moderate or low HBsAg titers, inactive or subsiding liver disease.
- Patients who are HBeAg + are often young, usually demonstrate elevation in serum aminotransferase, have high HBsAg titers, have active liver disease and remain very infectious.

Predisposing Factors to Developing Chronic Type B Hepatitis

- **Form of expression** of acute disease. Anicteric>icteric. Patients with an anicteric episode of hepatitis are more likely to develop chronic disease than those who have become jaundiced.
- **Age**
 1. Acutely infected neonates and infants develop chronic hepatitis B at an extremely high rate - 90-95% of infected neonates become carriers
 2. 20% of children become carriers
 3. Only 5-10% of adults who develop acute type B hepatitis will become chronic carriers.
- **Gender**
Women>Men
- **Immune Status.** Patients who have compromised immune systems are more likely to become chronic carriers. Examples include: hemodialysis patients and HIV patients.

Staging in Chronic Type B Hepatitis:

By definition, chronic type B hepatitis is the presence of HBsAg in the serum for more than six months. A **prognostic indicator** is the presence of *HBeAg in the serum for more than 10 weeks.*

- **Early Replicative Phase**
Characterized serologically by the presence of HBsAg, HBeAg, anti-HBc (IgM), as well as HBV DNA and DNA polymerase. In the liver one can demonstrate HBcAg, free HBV DNA and DNA polymerase.
- **Intermediate Phase**
Over a variable period of time, seroconversion of HBeAg into HBe antibody occurs. The annual rate is uncertain. Some reports suggest as little as 2.5% to as much as 25%. The clearance of HBeAg is usually followed by a 'window' period before anti-HBe can be detected. Usually this period does not exceed 1 year and may be shorter than 1 month. Coincident with this seroconversion is the decline in serum HBV DNA and DNA

polymerase activity. Circumstances do exist in which DNA polymerase activity and HBV DNA persist despite the absence of detectable HBeAg. It is known that just before HBeAg seroconversion some chronic carriers will undergo abrupt histologic and biochemical exacerbation of their liver disease. This phenomenon may be mistaken for an episode of acute type B hepatitis, particularly if the carrier state is unknown at the time of seroconversion, but can be differentiated by the absence of anti-HBc IgM antibodies.

- *Late Non-replicative Phase*
Characterized by the absence of HBeAg, the presence of HBe antibody, the absence of anti-HBc (IgM), HBV DNA and DNA polymerase from the serum.
In the liver, HBcAg may or may not be present, while HBV DNA is integrated into hepatic cells.
- *Resolution Phase*
The final stage of the carrier state is the disappearance of HBsAg. This is UNCOMMON occurring at the rate of 1-2% per year. These individuals generally develop anti-HBsAg and a return of normal liver function studies.

Liver Disease in an existing HBsAg carrier:

- *Reactivation of an existing chronic HBV infection*

Is not uncommon in:

1. Patients being treated chemotherapeutically for malignancies
2. Patients being treated with immunosuppressive agents after organ transplantation
3. Patients being treated with corticosteroids for a variety of disorders.
4. Some reports also suggest spontaneous reactivation in chronic carriers with a return of HBeAg, HBV DNA, and DNA polymerase. In one study this occurred in up to 30% of 25 HBsAg-positive patients who were anti-HBe positive and developed acute symptoms 4-13 months after seroconversion.

- *Superinfection of a chronic carrier with another hepatitis virus*
The chronic HBV carrier is often a member of another high-risk group subject to frequent and numerous viral exposures.

Examples:

1. Acute Hepatitis A virus in a chronic type B carrier:
 - a) [HAV (IgM) +, anti-HBcAg (IgM) -, HBsAg +].
2. Acute Non-A, Non-B or other injury in a chronic type B carrier:
 - a) [anti-HBcAg (IgM) -, HBsAg +].
3. Acute or chronic Delta hepatitis in a chronic type B carrier
 - a) [anti-HBcAg (IgM) -, HBsAg +, Anti-HD +].
 - b) Often mistaken for acute hepatitis B because of the presence of HBsAg., Delta superinfection often leads to a reduction in the concentration of HBV markers. The frequency of Delta infection in the United States among parenteral drug abusers and hemophiliacs is substantial.
 - c) Simultaneous acute type A and acute type B infection
 - i) Extremely rare. [HBsAg+, anti-HBc(IgM) +], HAV(IgM) +]

Chronic Type B hepatitis and Primary Hepatic Carcinoma (PHC):

Evidence supporting the role of persistent HBV infection in PHC was first suggested in the 1950s. In 1971, several additional studies showed a striking epidemiological association between HBV and PHC. The evidence includes:

- Carriers of HBV are common in the parts of the world where PHC is most common, including Alaska where HBV and PHC are common in the Eskimo population. In Taiwan, a prospective study of more than 20,000 male government workers begun in 1978 isolated 3500 asymptomatic HBV carriers. As of December 1983 116 cases of PHC had developed, 113 of these among HBV carriers. The lifetime odds of developing PHC or cirrhosis among asymptomatic HBV carriers in this group was between 40 and 50%.
- The virus can be identified by immunohistologic methods in the liver tissue of people with PHC. Curiously, the virus appears most abundant in the cells that do not appear to have undergone malignant transformation. The neoplastic cells often have little or no evidence of viral protein. The amount of HBsAg in patients with PHC is low compared to that of healthy HBV carriers and individuals with other forms of chronic HBV infection. The titer of HBsAg continues to decline as the PHC progresses.
- HBV DNA is integrated into the DNA of the host liver cells in a very large percentage of PHC cases, however, integration itself occurs in the liver cells of patients with chronic liver disease and asymptomatic carriers of HBV.
- The site of integration of HBV DNA is clonal (i.e. the same in every cell of an individual tumor), but the insertion location is not the same in different tumors.

Among the known causes for the occurrence of hepatocellular carcinoma (HCC), chemical carcinogens, chronic alcoholic intake and hepatitis B virus (HBV), especially in Asia, has been emphasized. HBV has been industriously studied and many queries about the relationship between HBV infection and hepatocarcinogenesis have been clarified. Recent discovery of hepatitis C virus (HCV) revealed that there might be the participation of this virus in hepatocarcinogenesis, as well. However, a precise mechanism in such a viral infection has not been known. Host immunological defense mechanisms may play a role. In addition, cellular gene abnormalities have been noted in the late period of cancer cell progression.

Hepatitis Delta Infection

Risk Factors for HDV infection:

In a 1986 study in Los Angeles, the most common behavior associated with Hepatitis Delta infection was *IV drug use*. In Los Angeles, 61% of a cohort of 154 patients with Hepatitis B infection had antibodies. Other related risk factors include homosexuality (13%) and combined homosexual practices and IV drug use (9.1%)

Who should be tested for HDV:

- High Risk Groups should be tested for possible HDV infection. These include:
 1. IV drug users
 2. Hemophiliacs
 3. Post-transfused patients
 4. Male homosexuals with multiple sexual partners
 5. Sexual partners of those in high-risk groups
 6. Hemodialysis patients
- Individuals from the Mediterranean and other areas endemic for HDV
 1. In general, with the exception of Southeast Asia where HBV is endemic, regions with endemic HBV also demonstrate endemic HDV.

Why Test for HDV?:

- It improves the therapeutic decision-making by clinicians.
 1. Some antivirals which are being used to decrease HBV replication (e.g. alpha interferon and adenine arabinoside (ara A)) are *contraindicated* in patients with co-existing HDV infection because these antivirals will increase viral replication.

- It allows better evaluation of the potential course of the disease
 1. Coexisting HBV/HDV infection has a more serious prognosis than HBV disease alone. It is more likely to be associated with fulminant hepatitis, chronic hepatitis and cirrhosis.
 2. There are reports that both infections together spread more rapidly within closed populations.