Blood and Blood Groups

Blood is a biologically active substance. It is the red body fluid that flows through all the vessels except the lymph vessels. Blood is a viscous fluid. It is thicker than water. Blood constitutes about 8 percent of the total body weight. The blood volume of an average sized male is 5 to 6 litres. The average sized female has 4 to 5 litres.

Blood is composed of two portions: formed elements (cells and cell like structures) and plasma (liquid containing dissolved substances). The formed elements compose about 45 percent of the volume of blood; plasma constitutes about 55 percent. Formed elements of the blood are of the following types: Erythrocytes (red blood cells); Leucocytes (white blood cells); Granular leucocytes (neutrophils / eosinophils / basophils); Agranular leucocytes (lymphocytes / monocytes); Thrombocytes (platelets). (Tortora and Anagnostakos, 1990)

Human red blood cell membranes contain over 300 different antigenic determinants, the molecular structures of which is dictated by genes at an unknown number of chromosomal loci. The term blood group is applied to any well defined system of red blood cell antigens controlled by a locus having a variable number of allelic genes, such as A, B, and O in the ABO system. The term blood type refers to the antigen phenotype, which is the serologic expression of the inherited blood group genes. Nearly all individuals produce “natural occurring” antibodies against the A or B
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antigens not present on their own red blood cells. In routine practice, the ABO type is determined by testing the red blood cells with anti-A and anti-B and by testing the serum against A, B and O red blood cells. (Giblett, 1991)

Levine and Stetson (1939) made the discovery of a new factor, when they observed that after a mother gave birth to a still born child and subsequently transfused with her husband’s blood she suffered a severe reaction in blood. Both the mother and the husband were group O, but they did not gave any name to this new factor. The name was given by Lansteiner and Wiener (1940), after its discovery. They conducted a study in which they injected blood from monkey-Rhesus Monkey into rabbits and guinea pigs, collected the blood, took the serum, which contain the anti-Rh factor, was mixed with the blood cells from a number of samples from individuals of a population of Newyork city. Red blood cells of 85% of this population agglutinated with this serum. This population was called rhesus-positive (Rh-positive). The remaining 15% that did not have any agglutination were rhesus negative (Rh-negative).

At least 30 commonly occurring antigens and hundreds of other rare antigens, each of which can at times cause antigen-antibody reactions, have been found in human blood cells, especially on the surfaces of the cell membranes. Most of them are weak and therefore of importance principally for studying the inheritance of genes to establish percentage. The particular groups of antigens are more likely than the others to cause blood transfusion reactions. They are the O-A-B systems of antigens and Rh- systems. (Guyton 2000).
Blood Donation and Donors

One of the keys to a good Blood transfusion is starting with good Blood. The most common eligibility guidelines of blood donor in the United States are outlined below.

- Be in generally good health and feeling well.
- Be at least 17 years of age; upper age 60 years.
- Weigh at least 110 pounds (45 kg).
- Pulse: 80 to 100 beats/min and regular.
- Temperature: Should not exceed 99.5 °F.
- Blood Pressure: acceptable range is 160/90 to 110/60.
- Skin: the venipuncture site should be free of any lesion or scar of needle pricks indicative of addiction to narcotics or frequent Blood donation (as in the case of professional Blood donors).

Arankalle (2000) reported that various organizations such as world health assembly, world health organization, international society of blood transfusion, international federation of blood donor organization and several national organizations have pleaded for voluntary blood donation as the universal standard. The data provided by Kapoor et al (2000) are alarming, as only 39% of blood donation in India seems to be voluntary. Thus the primary aim should be adequate donor motivation programmes. Potential donor populations need to be educated about the role of voluntary blood donations from individual without high risk behaviour.
Despite the fact that only 3.1% of blood donors in India are paid donors, the participation of so called "replacement donors" (58%) remains disturbing. The possibility of a significant proportion of these being paid donors can not be ruled out. If blood collected from such replacement donors is not tested or tested using inadequately sensitive serological assays, the possibility of infectious units being transfused is high.

Blood products intended for transfusion are routinely collected as whole blood (450 ml) in various anti-coagulants. Most donated blood is processed into components: Packed red blood cells (PRBC), Platelets and fresh frozen plasma (FFP) or cryoprecipitates. Whole blood provides both oxygen carrying capacity and volume expansion. It is the ideal component for the patients who have sustained acute hemorrhage of 25% or greater total blood volume loss. Whole blood is not readily available because of the need to process blood into components that can be used for different recipients, thus a continuous requirement for blood donation is needed.

Donor blood is transfused to the recipient, but sometimes transfusion leads to several complications. Transfusion reactions are classified as immune or nonimmune. The immunologically mediated reactions may be directed against red blood cells or white blood cells, platelets or at least one of the immunoglobulins, IgA. Other less well defined hypersensitivity reactions also occur. The major nonimmune reactions are due to circulatory overload, massive transfusion or transmission of an infectious agent.
Jeffery and Kenneth (1998) reported that infectious complications of transfusion have become less frequent, although fear of these complications remains the primary concern of both patients and clinicians. The incidence of transfusion related infections has been reduced substantially due to improved donor screening and testing of collected blood. Infections, like any adverse transfusion reaction, must be brought to the attention of the blood bank for appropriate "look back" studies. Infectious complications include: viral infections (hepatitis C virus, hepatitis B virus, human immunodeficiency virus, cytomegalovirus, human T lymphotropic virus type I, parvovirus B19), Bacterial contamination, parasitic infections (malaria, babesiosis, chagas disease).

Choudhury and Phadke (2001) reported that transfusion transmitted disease (TTD) is a major challenge to the transfusion services all over the world. The problem of TTD is directly proportionate to the prevalence of the infection in the blood donor community. In India, hepatitis B/C, HIV, malaria, syphilis, cytomegalovirus, parvo-virus B-19 and bacterial infections are important cause of concern. Hepatitis B and C infections are prevalent in India and carrier rate is about 1-5% and 1% respectively. Post transfusion hepatitis B/C is a major problem in India (about 10%) because of low viraemia and mutant strain undetectable by routine ELISA.
Hepatitis

**Mac Callum (1947)** reported in his study that viral hepatitis is a term reserved for infections of the liver by one or more of the distinct hepatitis viruses. The terms hepatitis A and hepatitis B were first introduced by him in 1947 in order to categorize infectious(epidemic) and serum hepatitis.

The terms hepatitis A and hepatitis B were eventually adopted by the world Health Organization Committee on viral hepatitis. *(WHO, 1973)*

**Schiff (1975)** reported that the first reference to epidemic jaundice has been ascribed to Hippocrates Flindt, a danish worker first put forward the hypothesis that the liver is the primary site for jaundice.

**Hepatitis B virus (HBV)**

**El-Serag and Mason (1999)** reported that hepatitis B viral infection is strongly associated with the development of hepatocellular carcinoma (HCC). Despite a declining incidence of HBV infection in the United States, the incidence of HCC has almost doubled in the last 15 years and mortality from HCC has increased by 41%, because of the large pool of individuals infected prior to the implementation of HBV immunization programs.

**Parkin et al. (2001)** reported that HBV infection is now the 5th most frequent cancer, killing 300000-500000 people each year.
Virology of HBV

Identification of an antigen that is intimately related to the causative agent of viral hepatitis has given impetus to hepatitis research. **Blumberg and associates (1965)** found this antigen incidentally while working on precipitins of beta-lipoprotein of all types. The precipitin was in the serum of a patient with haemophilia who had received multiple transfusions and the antigen happened to be in serum from an Australian aborigine. The precipitin line that formed in Ouchterlony double diffusion differed in specificity from known beta-lipoproteins, and the antigen was called Australia antigen or Au(1).

**Blumberg et al. (1967)** reported that a relationship was gradually recognized over the next 4 years between Australia antigen and leukaemia, Down’s syndrome, lepromatous leprosy and hepatitis and the possibility was considered that the Au(1) trait might be associated with susceptibility to viruses.

**Okochi and Murakami (1968)** confirmed the association between Au-Ag and hepatitis and demonstrated the appearance of antigen during the incubation period and acute phase of classic post-transfusion hepatitis.

**Prince (1968)** detected an antigen that reacted in the immunodiffusion test with serum from multiple transfused patients in the blood during the incubation period prior to the onset of major chemical or clinical
abnormalities. They identified an antigen termed SH in serum during the incubation period of a classical case of post-transfusion serum hepatitis. It has also been found in 7 out of 9 (6 of the latter undercode) additional cases of serum hepatitis and in 3 out of 2856 normal blood donors tested. Antibody to SH antigen was found in patients who had been multiply transfused such as patients with hemophilia and Cooley’s anemia. The antibody was not detected in sera from convalescent patients with typical cases of viral hepatitis.

**Bancroft et al. (1972)** compared the heterogenous antigenic determinants of hepatitis B antigen (HBAg) from 310 individuals by using a micro-Ouchterlony immunodiffusion technique: Reference antisera were made in rabbits immunized with a purified HBAg with ay determinants from an American with hepatitis and another purified HBAg with ad determinants from a Thai blood donor. They reported that the reference antisera were distinguishing two additional antigenic determinants, designated w and r. Data suggest w is more common than r on HBAg in the United States, but r is most common in Thailand.

**Le Bouvier et al. (1972)** reported that infection with type B hepatitis virus causes Australia antigen (HBAg) to appear in the serum. This antigen possess the “group” specificity, a, common to all HB Ag-positive sera; and one or other of two mutually exclusive subspecificities, d or y, which distinguish the two antigenic subtypes D and Y. These determinants d and y
reflect the activity of the two distinct genotypes of hepatitis B virus (HBV), provisionally designated HBV-D and HBV-Y. The study suggests significant differences in subtype distribution, such as the marked preponderance of Y in drug-abusers hepatitis.

**Nielsen et al. (1973)** determined the D(ad+, Y-) and Y (ay+, d-) subtype of Australia antigen in 199 antigen-positive patients with various liver diseases and 23 healthy carriers. In 183 consecutive patients with biopsy-verified acute viral hepatitis, the antigen was the Y subtype in 79 per cent and the D subtype in 21 per cent. 98 per cent of the drug addicts had the Y subtype whereas the D subtype predominated in the “Sporadic” Cases. The acute disease in the 183 patients with acute hepatitis was more severe in the group of the patients with antigen of the D subtype.

**Robinson et al. (1974)** gave the initial views of the hepatitis B genome through electron microscopy. In virions the genome appears to be circular, yet only partially double stranded.

**Gerlich and Robinson (1980)** reported that hepatitis B virus DNA contains a tightly bound protein which was not removed by heating to 60°C with 2% SDS, 2% mercaptoethanol. The protein was localized to a site near the 5' end of the complete viral DNA strand. It remained attached to this strand after heating with SDS to 90°C or treatment with 0.1 N NaOH, suggesting a covalent linkage. They suggested protein bound covalently to HBV DNA could be involved in the replication of the complete viral DNA strand and/or endonucleolytic generation of linear unit-length DNA pieces from replicative intermediates.
Chu et al. (1985) reported that the natural history of chronic HBV infection may be divided into three phases. The high replicative phase, characterized by HBeAg reactivity in serum and only minor histological activity, the low replicative (immune clearance) phase, during which the serum is positive for HBeAg or anti-HBe and histologic signs of chronic active liver disease are usually prominent, and finally, the nonreplicative phase, when the patient is anti-HBe positive and there is no evidence of inflammatory liver disease.

Cheng et al. (1986) reported that the envelope region of the hepatitis B virus (HBV) genome contains an open reading frame that begins upstream of the major surface protein gene. The two minor proteins that are initiated within the pre-S segment are immunogenic and may be involved in virus attachment to hepatocytes.

Chisari et al. (1986) concluded that inhibition of HBsAg secretion is related to a hitherto unknown property of the pre-S-containing domain of the large envelope polypeptide.

Eble et al. (1986) reported that hepatitis B surface antigen (HBsAg), the major coat protein of hepatitis B virus, is also secreted from cells as a subviral particle, without concomitant cleavage of N-terminal aminoacid sequence. They showed that the initial product of HBsAg biosynthesis is an integral transmembrane protein, with most or all of its C-terminal half on the lumenal side of the endoplasmic reticulum membrane. They concluded that
uncleaved signal sequences in p24 function to direct portions of the molecule across the membrane and are able to perform this function even when positioned in an internal protein domain.

**Pontisso et al. (1989)** reported that the surface antigen of hepatitis B virus (HBsAg) exposes three protein domains: preS1, preS2, and S. Pre S1 sequences expressed in transfected yeast cells bind specifically to plasma membranes of human liver. In the study they showed that purified virus particles from a virus carrier bind also specifically to such membranes. Subviral HBsAg filaments, which are rich in preS1, bind well too, while HBsAg 20-nm particles, which contain small amounts of preS1, bind to a much lesser degree. The binding can be inhibited by a monoclonal antibody, which recognizes a sequential epitope between amino acids 27 and 49 of the preS1 domain.

**Radziwill et al. (1990)** carried out a study to correlate the hepatitis B virus P gene with the enzymatic activities predicted to participate in hepadnavirus reverse transcription. The result suggests that the P protein consists of three functional domains and a nonessential spacer arranged in the following order: terminal protein, spacer, reverse transcriptase/DNA polymerase, and RNase H. The first two domains are separated by a spacer region which could be deleted to a large extent without significant loss of endogenous polymerase activity. The multifunctional P gene is expressed as a single translational unit and independent of the core gene and furthermore that the gene product is freely diffusible and not processed before core assembly.
Wu et al. (1990) reported the functional mechanism of the hepatitis B virus (HBV) X (HBx) gene product. The study suggests that the HBV-encoded trans-activator HBx is a novel protein kinase. Inactivation of the HBx protein by heat, protein-denaturing agents, or an ATP affinity analog, p-fluorosulfonylbenzoyl 5'-adenosine, resulted in loss of both protein kinase activity and trans-activation activity.

Raney and Lachlan (1991) reported that there are at least seven major subtypes of HBV distinguished by sequence difference in the surface antigen gene.

Lee (1997) concluded that hepatitis B virus is a small DNA virus that belongs to the family of hepadnaviruses. The viral genome consists of a partially double stranded molecule of about 3,200 nucleotides

Moradpour and Blum (2002) concluded that patients with HBsAg positivity for more than 6 months are termed chronic HBsAg carriers. Chronic HBsAg carriers can be divided into two groups, those with evidence of chronic liver disease and those without. The first group is defined as chronic hepatitis B, the second as asymptomatic or healthy HBsAg carrier state.

Replication of HBV (Figure 2)

Miller et al. (1984) reported that DNA-RNA hybrids may be intermediates in viral DNA replication and that the mechanism of hepatitis B
Figure 2. Schematic Diagram of The Life Cycle of HBV
virus (and closely viruses of ground squirrels and ducks) DNA replication differs from that known for other DNA viruses.

**Neurath et al. (1992)** reported the major target organ for hepatitis B virus is the liver. However, cells other than hepatocytes including peripheral blood lymphocytes and monocytes, may become infected with HBV. The cell receptor binding site was assigned to the preS(21-47) segment of the HBV envelope protein. The search revealed that interleukin 6 contains recognition sites for the preS(21-47) sequence and mediates HBV-cell interactions. Thus HBV belongs to a group of viruses utilizing cytokines or cytokine receptors for replication and interference with the host immune system.

**Lai (1995)** suggested that HBV possess a double stranded circular DNA genome that replicates by reverse transcription of an RNA pregenome.

**Natural History of HBV**

**Karayiannis et al. (1985)** examined the relationship of the presence of hepatitis B virus (HBV) DNA in serum, a measure of HBV-replication, to HBeAg/ anti-HBe status. In Northern Europe, there is a strong positive correlation between the presence of HBV-DNA and HBe antigenaemia and a negative correlation with the presence of anti-HBe. These associations are less marked in patients from Southern Europe, Africa, the Middle and Far East. When HBV-DNA is present in the serum of anti-HBe carriers, it is usually associated with the presence of severe liver disease or carcinoma.
Forty percent of patients with hepatocellular carcinoma had evidence of continuing HBV replication.

Tsang et al. (1986) carried out a study to correlate the presence of anti-HBs with immunity to subsequent infections with the hepatitis B virus. Over a period of 30 months, 64 patients with concurrently positive hepatitis B surface antigen (HBsAg) and antibody (anti-HBs) were identified at two institutions. When all assays were considered, 23.9% of HBsAg-positive individuals exhibited anti-HBs. In 10 patients, the subtype of HBsAg was ad, while the anti-HBs was anti-y. Concurrent HBsAg and anti-HBs is a pattern frequently observed throughout the spectrum of hepatitis B related events. The heterotypic antibody in these patients is of low titer, and its appearance or disappearance is not associated with changes in the clinical course; simultaneous HBsAg/anti-HBs positivity does not appear to reflect a distinct clinical entity.

Chu et al. (1995) studied the relationship between subcellular localization of HBcAg, liver inflammatory activity and hepatocyte regeneration in chronic hepatitis B, to test whether the dominant cytoplasmic expression of hepatitis B core antigen (HBcAg) in active chronic hepatitis B is secondary to liver damage and regeneration. The study suggests that following liver damage the regeneration of surviving hepatocytes might cause the shift of intracellular HBcAg from nucleus to cytoplasm. As a result, the extent of nuclear HBcAg expression reduces with concomitant increase in cytoplasmic HBcAg expression.
Immunology of HBV

Roggendorf et al. (1981) demonstrated antibodies of the immunoglobulin M (IgM) class against the hepatitis B core antigen (anti-HBc IgM) in acute and chronic hepatitis B. In a study of unselected patients whose sera were sent at irregular intervals for testing, anti-HBc IgM persisted in a high percentage (52%) for at least 13 to 18 months after onset of illness despite the fact that these patients eliminated hepatitis B surface antigen (HBsAg) and produced antibodies to HBsAg (anti-HBs). By using the anti-HBc IgM test as an additional aid in the diagnosis of acute HBsAg-negative hepatitis, the hepatitis B etiology could be established in 13 of 42 patients (31.4%).

Sheen et al. (1985) studied 376 patients with chronic hepatitis who were hepatitis B e antigen-positive for up to 7 yrs to examine hepatic decompensation associated with acute exacerbation preceding hepatitis B e antigen clearance in chronic type B hepatitis. Among the 165 patients who underwent hepatitis B e antigen clearance, 4 patients experienced hepatic decompensation and one of them eventually developed hepatic encephalopathy and died. The incidence of hepatic decompensation associated with hepatitis B e antigen clearance was 2.4%. Study suggest that such an event in previously unrecognized chronic hepatitis B carriers could have been erroneously interpreted as acute or subacute hepatic failure and that it might have been the result of a stronger enhancement of the host immune response.
Bodhiphala et al. (1999) carried out a study to detect HBV DNA in the HBsAg negative blood samples by using nested PCR with two primer pairs specific to core region. Two hundreds blood samples from HBsAg negative donors and 14 samples from HBsAg positive donors were provided by the blood bank of Ramathibodi Hospital. The results showed that HBV DNA was detected in all 14 HBsAg positive blood samples and in 7(3.5%) of 200 HBsAg negative blood samples. This study showed that the absence of HBsAg in otherwise apparently healthy individuals may not be enough to ensure lack of circulating HBV.

Jazayeri et al. (2004) reported that intracellular localization of hepatitis B core antigen (HBcAg) in vivo varies with liver cell damage. In hepatitis B e antigen (HBeAg) positive patients, HBcAg predominantly localized in the nucleus; in anti-HBe positive patients, it accumulated mainly in the cytoplasm. The study shows that the pattern of HBcAg localization in vitro depends on sequence and the serologic pattern of chronic infection, paralleling the situation in vivo.

Hepatitis B Genotyping

Ogawa et al. (2002) compared clinical manifestations and viral sequences of core, promoter and precore/core region among various genotypes of hepatitis B virus (HBV) in 25 patients with acute hepatitis. The genotype in patients with acute hepatitis was distributed differently from that
among chronic hepatitis patients in Japan, which are predominantly genotypes B and C. Of 25 patients with acute hepatitis, 14 had genotype A, five genotype B and six genotype C. Serum total bilirubin levels were significantly higher in patients with genotype A than in those with genotype C. Prothrombin time was shorter in patients with genotype B than those with genotype A or C. Total bilirubin was lower in patients with short duration of acute hepatitis. The serum ALT value remained above 1000 IU/l for over 10 days in 79% of patients with genotype A. The study showed the difference in the genotype little influenced the HBeAg/HBeAb phenotype in acute hepatitis patients. Thus understanding the viral genotypes in acute HBV infection may be valuable in predicting the clinical course of acute hepatitis B (AHB).

Epidemiology of HBV (Figure 3)

Dutta and Mahammed (1972) carried out a survey in 680 voluntary non-professional, 116 professional blood donors and in 174 proved cases of viral hepatitis using various techniques. 2.65 percent Au antigen positive samples were detected amongst voluntary blood donors and 2.58 percent amongst professionals.

Hill et al. (1973) used crossover electrophoresis to study the prevalence of Australia(Au) antigen carriers among blood donors. Over a 7 months period sera from 3398 individual donors were tested and 93 were
Figure 3. Prevalence of HBsAg Infections Among Blood Donors
shown to be Au antigen positive. The highest prevalence (3.8 percent) was
found among professional donors. Of patient relative donors and volunteer
donors, 2.3 percent and 0.75 percent respectively were shown to be Au
antigen carriers.

**Kelkar et al. (1973)** in a study of 420 persons in Pune, reported 1.49
percent HBsAg positivity.

**Kotwal et al. (1973)** reported 4.0 percent HBsAg positivity in
Aurangabad.

**Pal et al. (1973)** investigated a total of 1836 individuals for evidence
of serum hepatitis (SH) virus infection. Nineteen out of total 64 SH antigen
positive subjects were further followed up for varying periods with titration
of SH antigen in their blood samples. It was noted that 2.2 per cent of total
1001 adult male healthy voluntary blood donors as opposed to 0.43 per cent
of total 460 adult healthy females were carriers of SH antigen.

**De Stasio et al. (1976)** tested 3183 sera from healthy apulian
volunteer blood donors (18-65-year aged) by a commercial ria (ausria II
Abbott), for detection of HBsAg. The frequency of Australia antigen was
found to be 5.2% (168/3183) in all sera, 6.3% in male and 3.5% in female
donors, with differences statistically significant (p less than 0.01). In male
donors, the prevalence was highest in the first half of the life (18-40
years)(7.0%) than in the second half (41-65 years) (3.7%), and the difference
was also statistically significant (p less than 0.05). In female donors the
HBsAg frequency was 4.2% and 2.2% respectively for the first and the second half of the life, but the difference was not statistically significant.

**Babes et al. (1977)** investigated 457 healthy HBsAg carriers for ABO antibodies. They reported incidence of AB subjects was much higher among healthy carriers.

**Chakraborty et al. (1977)** reported 2.8 percent HBsAg positivity in Calcutta.

**Shanmugam et al. (1978)** tested 1600 sera in the southern region of Kerala by CEP and agar gel diffusion techniques and found the human carrier state of HBsAg to be 2.1 percent. Sera collected from pregnant women (3 percent), professional donors (2.4 percent) voluntary donors (1.5 percent) and healthy staff and students (0.6 percent) were found to be positive for HBsAg. A higher rate of HBsAg carrier state was found in group B donors (2.7 percent) while AB group donors were not found to be the carriers of the same. The carrier rate of HBsAg was higher in pregnant women (3 percent) and in the age group of 21 to 30 years (2.4 percent) while the same antigen was not detected in non-pregnant women and individuals above 40 years. Study points out the potential risk of post transfusion hepatitis and vertical transmission of HBsAg from asymptomatic carrier mothers to their children.

**Farzadegan et al. (1979)** compared the prevalence of HBsAg and anti-HBs among voluntary blood donors, professional blood donors, INBTS
laboratory staff, haemophiliacs and the patients and medical personnel of three haemodialysis centers in Iran. The 3.4% incidence of HBsAg found among 168,890 voluntary donors was significantly less than the 8.4% found among 378 professional blood donors. The prevalence of HBsAg was higher in male than in female donors, and also higher in single than in married donors. Prevalence of HBsAg was unrelated to ABO-Rh blood group but was related to age. Anti-HBs was found in 30% of voluntary blood donors, 67% of professional donors, 68% of haemodialysis patients, 39% of haemodialysis staff, 86% of haemophiliacs and in only 4.8% of HBsAg carriers. Subtyping of HBsAg found in 100 voluntary donors showed 65 were ay; 5 ad; 10 ad + ay and 20 were untypable.

Sobeslavsky (1980) studied 449 individuals and found 3.8 (CI 0.5-7.2) percent HBsAg positivity among 130 persons of 20-29 years age group, 7.4 percent (CI 2.6-12.1) HBsAg positivity among 122 persons of 30-39 years age group, 8.9 percent (CI 3.2-14.6) HBsAg positivity among 101 persons of 40-49 years age group, 6.3 percent (CI 1.3-11.2) HBsAg positivity among 96 persons above 49 years.

Lenka et al. (1981) studied 500 blood donors for the ABO blood groups and their relationship with Australia antigen (HBsAg). Most of the blood donors belonged to group B while the Australia antigen (HBsAg) was prevalent in the blood group A (9.30%). 0% frequency was found in AB blood group.
Shanmugham et al. (1981) tested 240 persons and reported 2.5 percent HBsAg positivity in Kerala.

Jayaprakash et al., (1983) tested 8085 volunteer donors, attending the blood bank at SCTIMST for hepatitis B surface antigen (HBsAg) carrier state by counterimmunoelectrophoresis and reported 103 (1.27%) were HBsAg positive. The personal data of donors showed a higher rate of HBsAg among men than women and in the age group of 21 to 30 years than in the other age groups. A significantly higher rate was noted among donors belonging to the lower socioeconomic group (P<0.05)

Prasad et al. (1983) reported 8.45 percent HBsAg positivity in Arunachal Pradesh.

Shanmugham et al. (1984) tested 10,600 persons and reported 1.3 percent HBsAg positivity in Kerala.

Tandon et al. (1986) reported that in Madras, 5.5 percent of voluntary blood donors, in Delhi 1.5 percent of 50,294 and in Chandigarh 1.0 percent of 13,839 voluntary blood donors were HBsAg carrier.

Baikie et al. (1989) carried out a study to determine the prevalence of the infection and to obtain a database to develop a vaccination strategy. Among five ethnic groups the HBsAg carrier rate was 3.2%. The HBsAg carrier rate was higher in males (male:female ratio 1.6:1.0). The rate of exposure to HBV was 4% for those below the age of 20 years and reached a peak for those aged 45 to 54 years.
Choudhary et al. (1989) reported 1.79 percent HBsAg positivity in Calcutta.

Makroo et al. (1989) carried out a study on Kashmiri blood donors for the prevalence of Hepatitis B surface antigen and reported that Eighty eight of 7900 healthy blood donors screened for hepatitis B surface antigen (HBsAg) carrier state by reversed passive haemagglutination assay were positive. The positivity was significantly more in rural donors (P<0.001) as compared to urban donors. False positive results are seen only with 1.13 percent of the sera tested.

Joshi et al. (1990) tested 1314 persons and reported 12.26 percent HBsAg positivity in Madhya Pradesh.

Singhavi et al. (1990) reported 2.16 percent HBsAg positivity in South India.

Thakur et al. (1990) tested 1012 persons and reported 3.26 percent HBsAg positivity in Himachal Pradesh.

Zekeng et al. (1990) screened 5,980 blood donors for Hepatitis B surface antigen (HBsAg) using the ELISA technique, in the University Teaching Hospital of Yaounde, Cameroon, between January 1988 and May 1989. In the study 11.72% of blood donors were found to be HBsAg positive.
Asakura et al. (1991) reported the seroprevalence of hepatitis B surface antigen (HBsAg) by gender and age, among blood donors at the Kitakyushu Red Cross Blood Centre Japan in the fiscal year of 1988. The positive rates of HBsAg among males were consistently higher than those of females, the peaks were detected in male donors aged 30-39 years and in females aged 40-49 years. Declining seropositive rates in individuals aged 50 years or over were observed for both genders.

Hussain et al. (1991) studied 227 persons and reported 24.5 percent HBsAg Positivity in Tamilnadu.

Martelli et al. (1991) screened 1,033 voluntary first time blood donors for hepatitis B infection in five blood banks in Goiana, Central Brazil, between October 1988 and February 1989. The survey was part of a major study designed to estimate seroprevalence of HBsAg and anti-HBs and to discuss methodological issues related to prevalence estimation based on data from blood banks. Donors were interviewed and blood samples were collected and tested for HBsAg and anti-HBs by ELISA tests. Prevalences of 1.9% and 10.9% were obtained for HBsAg and anti-HBs, respectively and no statistical difference was found between the sexes. Prevalence of anti-HBs increased with age (X2 for trend= 7.9 p=0.004).

Tsai et al. (1991) reported 4.5% HBsAg positivity in serum specimens of 1135 randomly selected voluntary blood donors in Taiwan.
Elavia & Banker (1992) reported 2.02 percent HBsAg positivity in Mumbai.

Emeribe and Ejezie (1992) screened 330 donors for HBsAg in Nigeria. They reported that blood group O donors had the highest HBsAg prevalence rate of 4.3% as against the 0% frequency for group AB donors. There were no significant association between ABO blood group distribution and the presence of HBsAg (P greater than 0.05).

Jayaram (1992) carried out a study to determine prevalence of HBV markers in different age groups in Madras and reported 4.5 percent (CI 1.6-7.4) HBsAg positivity among persons between 16-25 years, 5.9 percent(CI 2.9-8.9) HBsAg positivity among persons between 26-35 years, 6.4 percent(CI 2.0-10.8) HBsAg positivity among persons between 36-45 years, 3.9 percent(CI 0.0-9.2) HBsAg positivity among persons above 45 years.

Satoskar et al. (1992) tested 3104 blood donors in Mumbai and reported 4.7 percent HBsAg positivity.

Sumathy et al. (1992) reported 7.17 percent HBsAg positivity in a study of 530 persons in Vellore

Ahmed et al. (1993) screened 1239 normal donors from the Lagos University Teaching Hospital, Nigeria for ABO antibodies. Out of these 1239 donors, 220 (17.8%) were found to be in group A, 282 (22.8%) in group B, 85(6.9%) in group AB and 652(52.6%) in group O.
Khan et al. (1993) reported 2.4 percent of voluntary blood donors in Bangladesh are HBsAg carrier.

Thomas et al. (1993) carried out a study on health care personnel at the John Hopkins Hospital, Baltimore to determine the prevalence of HBV. They reported that antibodies to HBV core antigen were found in 59(6.2%) of 943 health care workers compared with 1879(1.8%) of 104,239 local blood donors (P < 0.001). Infection with HBV was associated with age (> or = 33 years) (P < 0.001), black race (P < 0.001), type of health care worker (nurse) (P = 0.02), 10 or more years of clinical employment (P = 0.003), and lack of HBV vaccination.

Aggarwal et al. (1994) reported that in India, 2-5 percent of voluntary blood donors are HBsAg positive.

Irshad et al. (1994) reported 2.6 percent HBsAg positivity in healthy persons in North India.

Mujeeb et al. (1994) reported that five percent of voluntary blood donors in Pakistan are HBsAg carrier.

Nagaraju et al. (1994) tested 605 persons and reported 9.9 percent HBsAg positivity with the help of Dot Blot technique in Lucknow.

Bar-Shany et al. (1995) carried out a study to compare age, sex and ethnic differences in the prevalence of hepatitis B surface antigen (HBsAg) among immigrant and Israeli-born blood donors. HBsAg was assayed by a
standard EIA in a sample of 136,977 blood donors in Israel during 1992. The overall age-adjusted prevalence of HBsAg was 0.85% in men and 0.44% in women.

Choudhury et al. (1995) tested 313 persons and reported 2.2 percent HBsAg positivity in Lucknow.

Panda (1995) found 2.5 percent HBsAg positivity in persons between 16-25 years age group, 3.7 percent HBsAg positivity in persons between 26-35 years age group, 4.9 percent HBsAg positivity in persons between 36-45 years age group and 6.0 percent HBsAg positivity in persons above 45 years in Delhi.

Zali et al. (1996) analysed the data obtained from the Survey of Health and Disease in the Islamic Republic of Iran with respect to hepatitis B-carrier epidemiology. Since the precise mode of transmission of hepatitis B was not well known, the study was designed to evaluate its transmission as a community-acquired disease. HBsAg tests were performed on the sera of 39,841 persons and the impact of several factors on the prevalence rate of HBV carriers was determined. The rate of hepatitis B carriers varied between zero and 3.9% with an average of 1.7%. Older males living in a village with low socioeconomic status, poor sanitation and intrafamily contact were the most important contributors to the rise of hepatitis B infection in the country.
Mohan et al. (1997) tested 1037 persons for the presence of HBsAg and reported 3.76 percent HBsAg positivity in Vellore.

Nanu et al. (1997) tested 1,32,093 voluntary and replacement blood donors and reported 2.5 percent HBsAg positivity in North India.

Rahlenbeck et al. (1997) screened 549 consecutive sera of male blood donors appearing at the blood bank of a regional hospital in Northwest Ethiopia, for the presence of hepatitis B surface antigen (HBsAg). Seroprevalence of HBsAg was found out to be 14.4%.

Lo et al. (1999) conducted a survey to collect transversal data to ensure a better understanding of the hepatitis B and C epidemiology in Mauritania. They have studied the seroprevalence rate of HBs antigen among 349 blood donors. Data of the study showed that HBs antigen was detected in 20.3% blood donors.

Mahoney (1999) reported that HBV infection accounts for 5,00000 to 1.2 million deaths each year and is the 10th leading cause of death worldwide. It is estimated that approximately 350 million people worldwide have chronic HBV infection and that 1 million persons die each year from HBV-related chronic liver disease.

Diouf et al. (2000) studied the prevalence of hepatitis B and C virus among chronic hemodialysis patients. The study concerned fifteen chronic hemodialysis patients in an unit of hemodialysis in Dakar. Only one patient
(6.7%) was HBsAg carrier while 9 patients (60%) were positive for antibodies to HBs without previous hepatitis B vaccine.

Ghavanini and Sabri (2000) determined the prevalence rate of hepatitis B surface antigen among 7897 healthy voluntary blood donors in Shiraz, Islamic Republic of Iran. Positive sera for HBsAg were found in 85 (1.07%) of the individuals.

Mujeeb et al. (2000) carried out a study to know the seroprevalence of HBV, HCV diseases among college going first time voluntary blood donors. Thirteen voluntary blood donors out of total 612 samples (2.21% with confidence limit 1.2-3.52%) were HBsAg positive. Seroprevalence of HBV infection among college going students is significantly low (<3.0%) than 30% seroprevalence among paid donors and 7% among family/replacement blood donors.

WHO (2000) reported that hepatitis B virus infection is a major global public health problem. Of the approximately 2 billion people who have been infected worldwide, more than 350 million are chronic carriers of HBV.

Bashwari et al. (2001) carried out a study on 57396 male potential blood donors to document the frequency of the ABO and rhesus blood groups. The study included a total of 19496 blood donors between the years 1985-1989 (referred as first period of study) and 37700 blood donors
between the years 1995-1999 (second period). ABO and rhesus blood groups from 200 Saudi females were also determined. The study shows the most common blood group was O (52%). The lowest blood group frequency was AB (4%). Rhesus Positive blood donors comprised 93% and Rhesus negative donors were 7%. Overall frequency of ABO and Rhesus blood groups were the following: O-positive 48%, A-positive 24%, B-positive 17%, AB-positive 4%, O-negative 4%, A-negative 2%, B-negative 1%, AB-negative 23%.

Butashvili et al. (2001) carried out a study to determine the prevalence of hepatitis B (HBV). Out of 4970 donors HBsAg was positive in 4.1% (3.4% confirmed).

Kaur et al. (2001) screened 60,780 voluntary blood donors in the state of Punjab for HBV infection by enzyme immunoassay and confirmed by Immunoblot assay. HBsAg seropositivity was found to be 1.7 percent.

Khan and Ahmad (2002) reported that in a series of 19,839 voluntary blood donors in Mumbai, 1.7 percent were found positive for HBsAg.

Luksamijarulkl et al. (2002) screened 2,167 blood donors for HBsAg, by Enzyme Immunoassay methods. The results revealed that prevalence of HBsAg positive among studied blood donors was 4.61%. When the prevalence was classified by selected socio-demographic
variables, it was found that variables including age, gender, marital status and occupation were significant for HBsAg positive rate (p=0.0068, p=0.0019, p=0.0048 and p=0.0017 respectively).

Thyagarajan et al. (2002) tested 1856 persons and reported 5.7 percent HBsAg positivity in Tamilnadu.

Gupta et al. (2004) carried out a study to find out the prevalence of HBsAg seropositivity in healthy blood donors. A total of 44064 blood units were collected in department of transfusion medicine, Dayanand Medical College & Hospital, Ludhiana. Screening of blood units was done by a fully automated microplate ELISA processor (ARIO model) from SEAC RADIM group using commercially available kits. HBsAg seropositivity was found to be 0.66% (290/44064).

Kim et al. (2004) reported the prevalence of chronic HBV infection in a U.S. community and describe demographic and clinical characteristics. The overall age- and sex-adjusted prevalence of HBV in this community was 0.15%. In a multivariable regression analysis, replicative status was the most influential (odds ratio [OR] = 5.98, P <.01) factor associated with abnormal aminotransferase values, followed by male gender (OR = 3.69) and age greater than 40 years (OR = 2.32 per decade).

Sakarya et al. (2004) reported 1.5% HBV prevalence in blood donors of Aydin region of Turkey.
Singh et al. (2004) analyzed all voluntary and replacement blood donors for the prevalence of infectious markers in East Delhi. A total of 52,500 blood units were collected and screened for hepatitis B surface antigen (HBsAg). Seropositivity was 1.8% (963/52,500) for HBsAg.

Akhtar et al. (2005) carried out an investigation to estimate the prevalence of HBV surface antigen (HBsAg) positivity, in asymptomatic volunteer male blood donors in Karachi, Pakistan. HBsAg prevalence in the male blood donors was found to be 2.0% (7048/351309).

Awaidy et al. (2005) screened 1710 pregnant women of Gulf States aged 15-45 years for the percentage of HBsAg positive and HBeAg positive individuals. A total of 7.1% of the women in Oman, 1% in Qatar and 1.5% in UAE were HBsAg positive. Risk factors identified for being HBsAg-positive were younger age, being a national (i.e. not an expatriate) and residing outside the city.

Dray et al. (2005) reported 10.4% seropositivity for HBsAg among 9006 volunteer blood donors at the National blood bank in the Republic of Djibouti.

Ramia et al. (2005) collected blood samples from 2505 Lebanese blood donors at random, at various periods of time at one blood donation center and screened for markers of HBV infection (HBsAg, anti-HBc and anti-HBs). The study showed HBsAg positivity of 0.6% and an overall exposure rate to HBV of 10.0%.
Tsatsralt-Od et al (2005) reported 8.2% seropositivity for HBsAg among 403 blood donors in Mongolia.

Transmission

Allen et al. (1962) studied factors influencing the frequency of occurrence of serum hepatitis from blood transfusions in a randomized sample of 21.4% of 11,627 patients who had received transfusions. Among patients who did develop hepatitis, the mortality rate was 0 for age groups less than 35 years old and 20% for age groups more than 40 years old.

Cherubin et al. (1970) tested the sera taken from patients with hepatitis who denied drug usage and from users of intravenous heroin and methamphetamine with and without clinical evidence of hepatitis for the presence of the serum hepatitis related antigen (SH) by an Ouchterlony technique. Study suggests either that the addiction problem is far greater than previously surmised or that other modes of transmission of SH antigen positive hepatitis exist in New York City.

Almeida et al. (1971) in their study showed an outbreak of Australia antigen positive hepatitis in a haemodialysis unit. The study suggests that outbreak was caused by airborne distribution of infected blood. Airborne droplets, containing virus, could be taken up either by ingestion or by a respiratory route.
Sterner et al. (1971) observed that four out of 5 Swedish naval recruits fell ill with overt serum hepatitis 9-11 weeks after having been tattooed immediately after each other by a London tattooer. Test for Australia antigen was carried out after 4 weeks of illness and later. Australia antigen was demonstrated in one patient. They reported that the rising frequency of serum hepatitis reported from different European countries will probably also increase the risk of contracting the illness after tattooing.

Hawkes et al. (1972) in his study observed that transmission of Au antigen by mosquito vectors could not be implicated in New Guinea. The overall incidence of Au antigen was 4.1% in the Bismarck range population and 2.6% in the Sepik population. Au antibody was not detected in any of the natives. There was no statistically significant difference between the frequency of the antigen in male and female natives. The incidence of Au antigen did not increase significantly with age.

Leevy et al. (1972) in his study showed that viral hepatitis may be transmitted by the mosquito. The study suggests that the mosquito can be a direct mechanical vector of viral hepatitis or serve as the source of infection if feeding is incomplete and the mosquito is killed while completing a meal on another subject.

Singleton et al. (1973) carried out a study to assess the risk of acquiring hepatitis B infection by “contact” with asymptomatic chronic
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carriers of hepatitis B antigen (HBAg). The study showed the following results: that the risk of acquiring hepatitis B infection from a household chronic carrier contact is related to the presence of hepatitis activity in that contact, sexual partners are at higher risk than other household contacts, and the household contact infectivity of chronic Hepatitis B antigen carriers with no evidence of hepatitis activity may be quite limited.

Heathcote et al. (1974) investigated 54 household contacts of 29 hepatitis B carriers for the presence of serum HBAg and antibody. The serological results showed that the contacts of carriers with evidence of liver dysfunction are at greater risk of presumed non-parenteral exposure to the HBsAg than are the contacts of “healthy” HBsAg carriers, as was most often demonstrated by the presence of anti-HBAg sometimes in high titre. Evidence of exposure to the HBAg was more common among the sexual partners than among the blood relatives of such carriers.

Johnson et al. (1974) reported in his study that seven of 48 (15%) young women with viral hepatitis had acquired the disease following earlobe piercing. The study suggests that the disease was contracted from the inadequately sterilized instruments used in the procedure.

Villarejos et al. (1974) examined feces, urine, saliva of chronic carriers of the hepatitis antigens and patients with acute Type B hepatitis by radioimmunoassay and counter immunoelectrophoresis to identify vehicles
of transmission of type B hepatitis Virus. The study showed that saliva is probably the main vehicle of infection in non-parenterally acquired Type B hepatitis. Transmission may be either airborne, through large droplets expelled by sneezing and coughing or directly from mouth to mouth by kissing or by exchange of chewed toys and candies among children.

Beasley et al. (1975) reported from his study of 147 babies born to mothers known to be carriers of hepatitis B surface antigen (HBsAg) that there is no evidence for a relationship between breast feeding and the subsequent development of antigenemia in the babies.

Alter et al. (1977) in their study inoculated chimpanzees intravenously with saliva and semen obtained from HBsAg positive individuals to assess the infectivity of hepatitis B surface antigen (HBsAg) containing body fluids other than blood. The study demonstrates that HBsAg positive saliva and probably semen contain infectious virus and suggests that saliva and semen may serve as important mechanisms in the transmission of type B hepatitis.

Beasley et al. (1977) in Taiwan reported the relationship of e antigen and it’s antibody (anti-e) to vertical transmission of hepatitis B surface antigen (HBsAg) from chronic asymptomatic HBsAg carrier women to their children.

Gilbert (1984) reported that many chronic carriers of hepatitis B virus acquire the infection from their mothers at birth; the risk is greatest if the
mother either has acute hepatitis B or is a hepatitis B e antigen (HBeAg) positive HBsAg carrier, but there is some risk also to the infants of mothers who do not carry HBeAg. At the Royal Women’s Hospital, Melbourne nearly 2% of women attending antenatal clinics were found to be carriers and without immunization, approximately five infants per 1000 infants delivered (up to 15 infants annually) also became carriers.

*Alter et al. (1989)* carried out a study to identify previously unrecognized sources for acquiring acute hepatitis B and non-A, non-B (NANB) hepatitis. The study suggests that heterosexual transmission may play an important role in the spread of Hepatitis B and NANB hepatitis.

*Chobe et al. (1991)* collected serum samples from dentists of Pune and students, staff, auxiliary staff and class D staff of a dental college in Bombay and conducted a study to assess the risk of hepatitis B infection among dental personnel. Dentists (32.02%), dental auxiliary staff (35.89%), clinical assistants and post-graduate students (19.56%) were found to have significantly higher prevalence of HBV infection. The infection was high among the dentists as compared to voluntary donors. The rate of increase in HBV seropositivity with age was higher (P<0.05) among dental personnel when compared to voluntary donors.

*Goldstein et al. (2002)* conducted an enhanced sentinel surveillance for acute hepatitis B from 1982 to 1998, in 4 counties in the United States to
determine trends in disease incidence and risk factors for infection. During 1994-1998, the most commonly reported risk factor for infection was high-risk heterosexual activity (39.8%) followed by MSM activity (14.6%) and IDU (13.8%).

**Stability of HBV**

*Bond et al. (1981)* reported in their study that however hepatitis B surface antigen (HBsAg) is very resistant to drying and relatively stable to heat. Moist heat at 98°C for 1 minute will inactivate HBV in a 1:10 serum dilution; the efficacy of moist heat at 60°C for 10 h varies with the HBV titre of the inoculum. The results indicate that if inanimate objects become contaminated with HBV (whether it be in blood or bodily wastes containing blood, plasma or serum) and are not properly cleaned and disinfected or sterilized, then these contaminated objects may contribute to disease transmission for periods of time up to one week and possibly longer.

**Diagnosis**

*Hoofnagle et al. (1978)* tested the hypothesis that donor blood containing antibody to hepatitis B core antigen (anti HBc) but lacking detectable hepatitis B surface antigen (HBsAg) and antibody to hepatitis B surface antigen (anti-HBs) might transmit type B hepatitis by examining donor and recipient serums from a veterans administration study of post transfusion hepatitis. The data stress the importance of anti-HBc as an
indicator of hepatitis B virus infection and support the hypothesis that high titer anti-HBc–positive blood might be infectious.

Stephen et al. (1984) reviewed serological test data from nine studies of hepatitis B conducted between 1980 and 1982 to determine the significance of certain serological test results commonly encountered in hepatitis B virus testing. Three tests, for hepatitis B surface antigen and for antibodies to hepatitis B surface antigen and hepatitis B core antigen (anti-HBs and anti-HBc), were used to measure hepatitis B virus infection risk in various populations. The findings indicated that presently available tests for anti-HBs and anti-HBc at low levels are often nonspecific and should be interpreted with caution.

Chung et al. (1993) evaluated 158 adult patients who received blood or blood products during open heart surgery in Hong Kong to compare the efficacy of various serological screening tests in the prevention of posttransfusion hepatitis. In hepatitis B virus-endemic areas, further reduction of the risk of posttransfusion hepatitis B with blood from HBsAg negative volunteer donors is difficult. The data suggest that excluding donors with isolated antibody to HBcAg may be the most effective approach.

Trepo et al. (1993) reported that hepatitis B virus serology has become extremely refined. As well as the recognized hepatitis B
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surface(HBs), hepatitis B core(HBc), and hepatitis B e (HBe) antigen-antibody systems, new markers have been introduced including pre-S1, pre-S2 for the envelope and the functional X protein. New automates have been introduced allowing flexibility in the different tests according to precise needs. The monitoring of pre-S1 antigen provides a relevant correlate of viral replication. The quantitative determination of HBV-DNA, pre-S1 Ag, and IgM anti-HBc seem most useful for the decision to use, and the monitoring of, antiviral treatment.

Recovery and Treatment of Hepatitis B

Realdi et al. (1980) reported that in HBsAg-positive chronic active hepatitis presence of HBeAg in serum identifies an early, although prolonged, phase of active virus replication that is frequently followed by the disappearance of complete virus particles from serum and appearance of anti-HBe.

Hoofnagle et al. (1981) observed that many patients with chronic type B hepatitis eventually have a spontaneous remission in clinical and biochemical evidence of active disease, usually heralded or accompanied by the disappearance of HBeAg and DNA polymerase.

Bonino et al. (1986) compared the clinical and serologic features of 118 hepatitis B surface antigen carriers with hepatitis B e antibody (anti-HBe) followed up for 3-10 yr (means 6.1 yr), separated according to the
presence or absence of hepatitis B virus-deoxyribonucleic acid (HBV-DNA) in serum. Carriers with serum anti-HBe/HBV-DNA had major liver disease; cirrhosis developed during the follow-up period in 9 of the patients. The replication of the hepatitis B virion terminated and liver disease remitted after seroconversion to anti-HBe. A positive HBV-DNA test in anti-HBe carriers is associated with a severe and evolutive liver disease and may provide an indication for treatment with drugs inhibiting the synthesis of HBV-DNA.

Perrillo et al. (1990) studied the efficacy of treatment with interferon. They reported in chronic hepatitis B treatment with interferon alfa-2b (5 million units per day for 16 weeks) was effective in inducing a sustained loss of viral replication and achieving remission assessed biochemically and histologically in over a third of patients. Moreover in about 10 percent of the patients treated with interferon, hepatitis B surface antigen disappeared from serum.

Wong et al. (1993) reported that Interferon had a significant treatment effect on the development of antibodies to HBsAg (anti-HBs), antibodies to HBeAg (anti-HBe) and on the normalization of alanine aminotransferase levels. They also suggested that Alpha-Interferon is effective in terminating viral replication and in eradicating the carrier state in patients with chronic HBV infection who are HBeAg positive when these patients are treated for 3
Follow-up studies are required to determine whether interferon reduces the risk for developing cirrhosis or hepatocellular carcinoma.

Josefson (1998) reported that interferon is administered through injection and causes prominent flu-like side effects, such as hyperpyrexia, myalgias, weakness, fatigue, anorexia, leucopenia and sometimes depression and hair loss. These factors limit its tolerability. Treatment with lamivudine, 100 mg a day for one year, resulted in regression in the severity of disease as measured by improvements in liver histology and decreases in the levels of transaminases. Overall, 52% of patients taking lamivudine showed significant histological improvement compared with 36% of those taking interferon and 25% of those taking placebo. Side effects noted with lamivudine treatment included malaise or fatigue; nausea and vomiting; and headache and abdominal pain. The frequency of these side effects was not significantly greater than with placebo.

Xiong et al. (1998) studied the inhibition constants of adefovir diphosphate and lamivudine triphosphate for wild type and mutant human HBV DNA polymerases, which contain amino acid substitutions associated with lamivudine resistance, to determine whether adefovir is active against lamivudine-resistant hepatitis B virus (HBV). HBV DNA polymerase mutants M5521, M552V, and L528M/M552V showed resistance to
lamivudine triphosphate with inhibition constants (Ki) increased by 8.0 fold, 19.6 fold, and 25.2 fold compared with that of the wild type HBV DNA polymerase. However, these mutants remained sensitive to adefovir diphosphate with the inhibition constants increasing by 1.3 fold and 2.2 fold or decreasing by 0.79 fold. The L528 M single mutation, identified in patients with increasing HBV DNA levels during therapy with famciclovir, also remained sensitive to adefovir diphosphate with the inhibition constant increased by only 2.3 fold.

**Cho (2004)** reported that the lowest serum HBV DNA levels during lamivudine therapy is the predictable factor for the viral breakthrough in patients with chronic HBV infection.

**Clawson et al. (2004)** developed the SNIPAA cassette, which contains a double-dose of a special type of ribozyme called a trans-acting hammerhead ribozyme. Ribozymes are ribonucleic acid (RNA) segments that, like enzymes, cause chemical changes or splitting in other RNA segments. RNA, which is critical to the replication of DNA—life's instruction book—also is critical to the replication of viruses. The SNIPAA cassette was packaged in liposomes, typical vehicles for delivering drugs in the body, and the liposomes in turn were modified with proteins so that they would seek out the liver cells where the HBVs replicate. Once at the liver cell, the SNIPAA cassette package is released into the cell and finds its way to the cell nucleus, where the active ribozymes are produced. The ribozymes
destroy the viral RNA’s ability to produce proteins by cleaving, or cutting, the viral RNAs rendering them useless. Proteins are critical to virus replication. They recorded a greater than 80 percent reduction in the HBV liver DNA.

Schiff (2004) reported Entecavir(ETV) is an antiviral with potent, selective activity against wild type and lamivudine resistant HBV. At 0.5 and 1.0 mg doses, ETV has demonstrated superior antiviral activity in a phase II dose ranging study of ETV(0.1, 0.5, 1.0 mg daily) versus continued LVD in LVD-refractory patients.

Zonneveld (2004) in his study analyzed viral decline in 36 HBeAg positive chronic hepatitis B patients (who completed 24 weeks of PEG-IFN monotherapy or combination therapy), to evaluate viral dynamics during therapy with pegylated interferon alfa-2b/ Peg-IFN(Peg-Intron) and to establish if combination therapy with lamivudine(Epivir-HBV) has an additive effect. In the combination therapy group they found a biphasic decline of HBV-DNA. The efficacy of combination therapy was 94.9%. In this group HBV-DNA decline during the first week was associated with response. In the monotherapy group a more complex HBV-DNA decay pattern was found, not fitting a biphasic model. In this group a typical staircase pattern with minimal decline in the first weeks but a sudden HBV-DNA decrease after 12 to 20 weeks was found in 6 of the 8 responders (75%).
Prevention and Vaccination for Hepatitis B

Mulley et al. (1982) examined the cost effectiveness of three strategies: vaccinating everyone, screening everyone and vaccinating those without evidence of immunity and neither vaccinating nor screening but passively immunizing those with known exposure to formulate indications for the use of hepatitis B vaccine. Study showed that screening followed by vaccination of homosexual men and vaccination without prior screening of surgical residents would result in savings of medical costs. Neither screening nor vaccination is the lowest cost strategy for the general population. Vaccination of susceptible persons will save medical costs for populations with annual attack rates above 5 percent. Vaccination may be considered cost effective (or cost saving when indirect costs are included) for populations with attack rates as low as 1 to 2 percent.

Jilg et al. (1988) studied antibody levels to hepatitis B surface antigen (anti-HBs) in healthy adults vaccinated with three doses of plasma-derived hepatitis B vaccine, containing 20, 10 or 5µg of the antigen, for 4-6 years. Four years after the first vaccination, anti-HBs levels in 34% had dropped below 10 IU/l. The persistence of anti-HBs above this value depended on the peak antibody response after the third vaccination. Whereas all vaccines tested with peak anti-HBs levels above 10,000 IU/L still had levels above 10 IU/L after 6 years, no-one with initial values between 10 and 100 IU/L
maintained antibody concentrations above 10 IU/L for longer than four years. The rate of decrease in anti-HBs was independent of the peak anti-HBs value, the vaccine dose and the age and sex of the vaccinees.

Garman et al. (1990) reported that in Southern Italy, 44 contacts of hepatitis B virus carriers, including infants of carrier mothers, became HBsAg positive despite passive and active immunization according to standard protocols. In 32 of these vaccinees infection was confirmed by the presence of additional markers of viral replication. In infant, serious disease occurred. The virus from this patient was an escape mutant with a different sequence from that of the isolate from the mother. A point mutation from guanosine to adenosine at nucleotide position 587 resulted in an amino acid substitution from glycine to arginine in the highly antigenic determinant of HBsAg. This mutation was stable. It was present in an isolate from the child 5 years later. In some of these patients, including this child the a determinant to which a large part of vaccine induced immunity is directed, has been partly lost.

Geissler et al. (1998) studied the effect of co-immunizations with cytokine DNA expression constructs encoding for interleukin(IL-2) and (GM-CSF) on the immunogenecity of large envelope protein (LHBs) at the B- and T-cell level. They reported that genetic co-immunization of HBV middle envelope protein(MHBs), but not (LHBs), with IL-2 or GM-CSF DNA expression plasmids augments helper T-cell, CD8+ CTL and humoral
immune responses to HBV envelope proteins and therefore may be an interesting approach to treat chronic HBV infection.

Mahoney (1999) reported that acute and chronic hepatitis B virus (HBV) infection is a leading cause of liver disease worldwide. It is estimated that approximately 350 million people worldwide have chronic HBV infection and that 1 million persons die each year from HBV-related chronic liver disease. In the past decade, effective treatment modalities have been developed for persons with chronic infection. The Global Advisory Group to the World Health Organization recommended that all countries integrate hepatitis B vaccine into national immunization programs. Recent reports from Taiwan indicate a reduction in the incidence of liver cancer among children as a result of widespread hepatitis B vaccination programs.

Sanchez-Tapias et al. (2004) reported that most western countries have a low endemicity pattern of hepatitis B virus (HBV) infection. However, the penetrance of this disease is quite variable, from very low in some areas to intermediate or very high in others. In recent years, improvement of social conditions, observation of universal precaution measures, and implementation of different immunization strategies has led to significant changes in the epidemiology of this infection. There is an ongoing substantial decline in the incidence of acute hepatitis B, particularly in children and young adults but less significant in older persons, who are not yet sufficiently covered by current preventive strategies.
Hepatitis C Virus

The third national health and Nutrition Examination survey (NHANES-III) performed by the national Institutes of Health (NIH) between 1994 and 1998 estimated that 3.9 million people or 1.8% of the population in the United states are Hepatitis C antibodies positive, while 2.7 million patients are truly infected with the virus and thus have the risk of developing the consequences of this infection. (NIH 1997)

Badur and Akgun (2001) reported that worldwide viral hepatitis is still recognized as a major problem particularly in developing countries. During the past two decades there has been important progress in the field of viral hepatitis; the adaptation of molecular biology techniques to viral hepatitis has proven to be of great utility in the diagnosis of 'classical' hepatitis viruses, in monitoring during treatment, and also in learning more about the 'new' viruses.

According to World health organization (2002), 3% of the world’s population, or approximately 170 million people are infected with hepatitis C virus.

General Virology of HCV (Figure 4)

Bradley et al. (1985) estimated the size of the virion to be 180 nm by ultra centrifugation.
Figure 4. Model of The Human Hepatitis C Virus
He et al. (1987) suggested the size of the virion to be 30±60nm by his filtration studies.

Bukh et al. (1995) reported that apart from differences in length HCV genotypes show diversities of around 30% in the nucleotide sequences of their whole genomes and comprehensive analysis of these sequences has revealed the existence of at least 6 genotypes and more than 30 subtypes throughout the world.

Chamberlein et al. (1997) reported that the viral genome is a single stranded RNA molecule approximately 9±5 kb in length which is a positive sense and possesses a unique open reading frame, coding for a single polyprotein flanked by untranslated regions at both it’s 5’ and 3’ ends. The length of polyprotein encoding region varies according to the isolate and genotype of the virus from 3008 to 3037 amino acids. (Figure 5)

5’ Untranslated Region (5’UTR)

Jackson et al. (1990) reported that there are some sites in the 5’ UTR region of the genome allowing ribosomes to bind internally on the genome and initiate translation at specific AUG codons

Han et al. (1991) reported that an obvious characteristic of HCV is the presence of a long untranslated region (UTR) at the 5’ end of the genome and detailed molecular analysis indicates that polyprotein synthesis is initiated at nucleotide 342.
Figure 5. Hepatitis C Viral Genome
Fukushi et al. (1994) analysed the model of the structure of the HCV 5’ UTR proposed by Brown et al (1992) and indicated that a stem-loop structure is formed at nucleotide 46 which subsequent deletion mutagenesis experiments have shown to be critical for Internal ribosomal entry sites (IRES) function.

Smith et al. (1995) reported the long Untranslated region to be the most conserved region of the whole genome, a characteristic which has allowed it to be used as a diagnostic marker for HCV by PCR.

Virus Encoded Proteins

Hijikata et al. (1991) reported that the nascent viral polyprotein is processed by a combination of host & viral proteinases into the mature viral proteins. At least 10 distinct viral proteins have so far been identified which are arranged so that structural proteins located in the amino-terminal one-third and the replicative enzymes located within the carboxy terminal two-thirds of polyprotein.

Core

Ralston et al. (1993) reported that the protein located at the amino terminus of the polyprotein is highly basic in nature and is considered likely to be the viral capsid protein. It is released from the viral polyprotein by nascent proteolytic cleavage at amino acid 191 by host proteases. The full length protein known as P21, has been identified by both in vitro and in vivo expression.
Santolini et al. (1994) reported a second species (P19) generated by a secondary cleavage at amino acid 173, is the major product observed following expression in mammalian cells. Both P21 and P19 are located in the endoplasmic reticulum (ER) membrane and the conversion of P21 to P19 is presumably mediated by membrane associated cellular enzymes.

Lo et al. (1995) reported a third collinear species of core which can also be detected in expression studies, is approximately 151 amino acids long (P16) and appears to be localized in the nucleus and more specifically in the nucleolus. In particular the nucleolar localization of P16 may be due to its ability to bind to ribosomes, which are assembled in the nucleus.

Barba et al. (1997) showed an association between the core protein and the surface of lipid droplets within the cytoplasm. Analysis of the triglyceride populations within the cell indicates that core protein expression stimulates a change in cellular metabolism of triglycerides. Since a characteristic of HCV infection is liver steatosis it is plausible that this occurs as a result of the direct effect of the core protein on lipid metabolism.

E1/E2

Weiner et al. (1991) reported that E2 represents the most variable region of the HCV genome.

Grakoui et al. (1993) reported that the major viral structural proteins are the glycoproteins E1 and E2 which are released from the viral
polyprotein by the action of host cell signal peptidases. They analysed the amino termini of E1(gp35) and E2(gp70) and indicated that both are cleaved at amino acids 383 and 746 respectively.

Kumar et al. (1993) reported that within the E2 sequence are regions of extreme hypervariability (HVR) which have been the focus of more detailed study and one of these regions known as HVR-1 represents the amino-terminal 34 amino acids within E2, spanning residues 383±414. This region has been suggested to be particularly important in HCV neutralization because of it’s extreme variability and the fact that this variability was not observed in a patient with agammaglobulinaemia even over a period of 2±5 years.

Miyamura and Matsuura (1993) reported that both proteins E1 and E2 are heavily glycosylated.

Dubuisson et al. (1994) analyzed the E1-E2 complexes and showed that the non-covalently linked forms were slow-forming stable species thus rate limiting step in the process while the disulphide linked forms were rapidly forming misfolded aggregates.

Mizushima et al. (1994) reported that E2 is sometimes found extended at it’s carboxy terminus to include a smaller protein known as p7. Unlike the other cleavages within the structural region, proteolytic cleavage between E2}p7 and p7}NS2 appears to occur post-translationally.
Dubuisson et al. (1996) showed the efficient folding and assembly of the heterodimeric E1-E2 complexes to occur within the Endoplasmic Reticulum and is dependent on an initial prolonged association with the chaperone calnexin.

Deleersnyder et al. (1997) reported E1-E2 complexes slowly mature into non-calnexin bound complexes.

**Non structural Proteins**

Santolini et al. (1995) showed the NS2 protein to be a transmembrane protein with its carboxy terminus translocated into the lumen of the ER while its amino-terminus lies in the cytosol.

Enomoto et al. (1996) suggested apart from the probable role of NS5A in the replication cycle, it may be a critical factor in determining the susceptibility of the virus to treatment with IFN. It was initially reported that IFN sensitivity correlated with mutations within a discrete region of NS5A which was subsequently named the IFN sensitivity determining region (ISDR).

Kim et al. (1996) showed that NS4A forms an important structural feature of the active NS3}4A complex which acts as a protease.

**3’ Untranslated region (3’ UTR)**

Takamizawa et al. (1991) suggested that the 3’ terminus of the genome terminated in a poly (U) tract.
Natural history of Hepatitis C (Figure 6)

Alter et al. (1992) reported that patients with community acquired hepatitis C have a high rate of chronic hepatitis. HCV may be a major cause of chronic liver disease in the United States, and in most patients HCV infection seems to persist for at least several years, even in the absence of active liver disease.

Seeff et al. (1992) reported that Cirrhosis may take many years to develop thus HCV infection may be silent for decades before the appearance of features of portal hypertension, hepatocellular failure, and hepatic encephalopathy.

Takahashi et al. (1993) reported that wide variation in the severity of hepatocellular injury in persistent HCV infection suggests a progressive pattern of disease, where mild hepatitis can eventually develop into CAH and ultimately cirrhosis.

In addition to Hepatic disease, there are important extrahepatic manifestations of HCV infection.

(1) Mixed cryoglobulinaemia. (Agnello et al., 1992)
(2) Porphyria cutanea tarda. (Fargion et al., 1992)
(3) Sjogren’s syndrome. (Haddad et al., 1992)
(4) Membrano proliferative Glomerulonephritis. (Johnson et al., 1993)
(5) Lichen Planus. (Tanei et al., 1995)
Female sex, young age at infection

(Slow)

≥30 years

Normal liver → Acute infection → Chronic infection develops in 80% → Chronic hepatitis → Cirrhosis develops in 20% → Risk of carcinoma, 1–4% per year

(<=20 years)

Alcohol use, coinfection

Figure 6. Natural History of Hepatitis C
Goodman and Ishak (1995) reported two categories still widely used to describe the severity of chronic hepatitis are chronic persistent hepatitis (CPH) and chronic active hepatitis (CAH). CAH is characterized by piecemeal necrosis and the presence of inflammatory infiltrates in the liver parenchyma & is considered a progressive disease process that leads eventually to cirrhosis. In addition to necroinflammatory lesions, hepatitis C is often characterized by damage to bile duct epithelium, accumulation of fat vesicles (steatosis) and lymphoid aggregates in portal tracts.

Kolykhalov et al. (1997) reported that more than 1% of the world’s population is chronically infected with hepatitis C virus (HCV). HCV infection can result in acute hepatitis, chronic hepatitis and cirrhosis, which is strongly associated with development of hepatocellular carcinoma. Genetic studies of HCV replication have been hampered by lack of a bona fide infectious molecular clone. Full length functional clones of HCV complementary DNA were constructed. RNA transcripts from the clones were found to be infectious and to cause disease in chimpanzees after direct intrahepatic inoculation. The work defines the structure of a functional HCV genome RNA and proves that HCV alone is sufficient to cause disease.

Immunology of HCV

Zibert et al. (1995) reported that serum antibodies from chronically infected subject also block binding of fusion protein that only contain
sequences of HVR-1, a 34 amino acid domain located between amino acid 384 and 414 of E2, which is the most variable region in the HCV genome. Strain specific antibodies can also be detected in the serum of chronically infected subject.

Pileri et al. (1998) reported that serum antibodies that neutralize the infectivity of HCV for chimpanzees and established human cell lines are present during persistent infection. Epitopes recognized by these neutralizing antibodies have not been identified, but are thought to be contained primarily in the E2 envelope protein. E2 also contains the binding site for CD81, a tetraspan expressed on hepatocytes and B lymphocytes that is thought to function as a cellular receptor or co-receptor for the virus.

Leechmann et al. (1999) carried out a study to investigate the T cell response to hepatitis C virus core and core derived antigens. The data suggest that patients with persistent viremia and chronic liver disease (group B) have less peripheral blood mononuclear cell (PBMC) showing type 1 cytokine (IL-2, IFN-γ) responses to HCV core protein than patients with self limited HCV infection (Group A).

Walker (1999) reported that seroconversion to HCV antigens typically occurs several weeks to months after infection. Chronically infected subjects have antibodies against most HCV proteins, but persistent viremia might occasionally occur without anti-HCV antibodies. There are no
clear-cut serologic markers distinguishing chronically infected subjects from those who resolve disease, except perhaps HCV – specific IgM antibodies that appear to be a maker of viremia.

Mc Caugham (2000) reported that during chronic HCV, there seems to be a chronic elevation of the cell mediated immune response with little fluctuation during the course of the illness. Visual level do not drop dramatically during ALT flares and whenever the liver is sampled in this condition, T1 immune responses, activated cytotoxic T Lymphocyte (CTL) responses and cell mediated immune (CMI) responses are seen.

**Epidemiology of HCV (Figure 7)**

Sirchia et al. (1990) tested 24 blood transfusion services evenly distributed throughout the various Italian regions for the presence of hepatitis C virus (HCV) antibodies in the serum and serum alanine aminotransferase(ALT) level. The study showed anti-HCV seroprevalence in Italy was 0.87% with a difference between Northern and Southern regions (.68 vs. 1.37%) and between younger and older subjects(0.62 vs. 1.21%)

Lee et al. (1991) conducted a study on comprehensive seroepidemiology of hepatitis C infection in Taiwan by using first generation ELISA. Study showed that among normal population, zero percent of 1000 individuals under the age of 20 years, 0.6 percent of 338 aged 21 to 40, and 2.5 percent of 81 aged over 40 were anti-HCV positive.
Figure 7. Prevalence of HCV Infection Among Blood Donors
For the high risk groups, the seropositive rates were: 100 percent of 9 hemophiliac patients, 53 percent of 115 intravenous drug abusers, 34.4 percent of 96 hemodialysis patients, 15.8 percent of 19 HIV positive homosexual men, 7.1 percent of 196 prostitutes, 5.9 percent of 34 spouses of anti-HCV positive patients.

**Tsai et al. (1991)** tested serum specimens of 1,135 randomly selected voluntary blood donors for antibodies to HCV by Ortho enzyme-linked immunosorbent assay. A total of 18 donors were found to be positive for anti-HCV with a prevalence of 1.6%. Females had a higher prevalence (11/491 = 2.2%) than male (7/644 = 1.1%). The prevalence of anti-HCV for age groups of 18-30, 31-45, and 46-60 years was 2.0%, 0.8% and 0.0%, respectively for females. The HBsAg positivity rate was 4.5% in all specimens tested. There was no significant correlation between HBV and HCV infections. Although those who had a history of surgical operation, tattooing and ear piercing had a higher anti-HCV prevalence than those without such a history (2.8% vs 1.2%, 4.0% vs 1.5% and 2.1% vs 1.5% respectively), the difference were not statistically significant.

**Darwish et al. (1992)** carried out a study on 90 serum samples from non-professional blood donors to find out the prevalence HCV among those blood donors. Results demonstrated overall positivity rate of 14.4% for anti-HCV by RIBA test. The percentage of reactive sera was 6% for the age
group of 20 to below 30 years and 37.5% in those aged above 30 years and this difference was statistically significant.

Aymard et al. (1993) studied the prevalence of hepatitis C antibodies in 60,960 blood donors from the north east of France. Using a second generation ELISA, 424 donors (0.69%) were reactive, with no significant difference between males (0.69%) and females (0.70%).

Darwish et al. (1993) performed serologic tests for hepatitis C virus infection on sera obtained from 163 volunteer blood donors seen at one Cairo hospital. They found HCV infection in 36 donors (22%) measured by a second generation enzyme immunoassay. Thirty-five of these 36 positive sera were tested with a second generation recombinant immunoblot assay (RIBA-2); 22 (63%) were reactive and another 12 (34%) showed an indeterminate reaction. Overall, 13.6% (95% confidence interval [CI] = 8.3-18.9%) of these Egyptian blood donors were serologically confirmed to be infected with HCV.

Thomas et al. (1993) reported that antibodies to HCV were found in seven (0.7%) of 943 health care workers and 0.4% of local blood donors at the John Hopkins Hospital, Baltimore.

Yano et al. (1993) reported that 2.3 million (1.9 percent) Japanese among the 121 million were anti-HCV positive by the second generation assay. The prevalence in the age group less than 20 was found to be 0.2
percent, in 21-30 years was 0.7 percent, in 31-40 years was 1.9 percent, in 41-50 years was 2.6 percent and in age group above 50 was found to be 3.9 percent.

**Hernandez-Perez et al. (1994)** reported that the main transmission mechanism for hepatitis C virus is through blood products. In order to know seroprevalence of antibodies in military personnel, 2,564 samples at the Central Military Hospital with a second generation Enzymatic Immunoassay (EIA-2) were studied. All participants were males; the mean age was 25 years (range 17-47). Positive results were found in 19 potential donors (0.74%).

**Ilas et al. (1994)** performed a survey to assess the hepatitis C virus seroprevalence in volunteer blood donors of the National Medical Centre, Hospital de Especialidades, Mexico City. Serum samples from 1100 individuals were collected. Second generation enzyme linked immunosorbent assay test was used for the screening. The antibodies against hepatitis C virus (anti-HCV) in the volunteer blood donors were positive in 0.7%.

**Merino-Conde et al. (1994)** showed the seroprevalence of anti-HCV in candidates for blood donation at the General Hospital of Mexico. 330 individuals were studied. Determination of anti-HCV was performed by the UBI HCV EIA diagnostic test. Risk factors such as history of major surgery
, transfusions, drug addiction, etc were also assessed. There were only 4 seropositive patients (1.2%).

Araj et al. (1995) performed a study to determine the prevalence of HCV antibodies in 536 random Lebanese blood donors using three enzyme immunoassay kits. The overall prevalence of HCV antibody in these blood donors was 0.7%.

Sulaiman et al. (1995) investigated 7,572 blood donors from 21 of the 27 provinces and reported the overall anti-HCV positive rate was 2.1 percent. The study showed a marked increase from 1.1 percent among those 10 to 40 years old to 4.1 percent among the group aged 41 to 50 and 10.3 percent those above 50 years. No difference was found between males and females or among different locations.

Sun et al. (1995) reported from a survey on 433 plasma donors and 461 whole blood donors from 4 county plasmapheresis units and 5 city blood centers in Hebei province, an alarming 55.5% and 10.2% were anti-HCV positive respectively. One year after the introduction of anti-HCV screening that eliminated the known anti-HCV positive donors, 793 plasma donors and 873 whole blood donors in 3 city blood centers and 4 county hospitals were again surveyed. Anti-HCV positive rate had reduced dramatically to 4 percent and 0.2 percent respectively. All the 86 first time donors were negative for anti-HCV.
Wang (1995) reported that seroprevalence of anti-HCV among blood donors in Singapore was 0.37 percent. Analysis of 241 seropositive blood donors showed a higher prevalence in male than female, 0.389 percent and 0.298 percent respectively. Significant differences were also seen among the races that is 0.329 percent in Chinese and 0.513 percent in Malay.

Jaiswal et al. (1996) reported that in Central India, 1.8 percent of the 289 voluntary blood donors were anti-HCV positive.

Murphy et al. (1996) screened total of 862,398 consecutive voluntary blood donors and showed that there were 3126 donors with at least one blood donation confirmed HCV-seropositive, for a crude prevalence of 3.6 per 1000. Age-specific HCV seroprevalence rose from 0.5 per 1000 donors younger than 20 years to a maximum of 6.9 per 1000 in donors aged 30 to 39 years and declined in older age groups. There was interaction between age and educational attainment, with 30 to 49 year olds with less than a high school diploma at highest risk of HCV infection (odds ratio[OR], 33.0; 95% confidence interval[CI], 23.0 to 47.2 compared with those younger than 30 years with a bachelor's degree or higher degree). Other independent risk factors for HCV seropositivity included male sex (OR, 1.9; 95% CI, 1.8 to 2.1), Black race (OR, 1.7; 95% CI, 1.6 to 1.9), Hispanic ethnicity (OR, 1.3; 95% CI, 1.1 to 1.5), previous blood transfusion (OR, 2.8; 95% CI, 2.5 to 3.1), and first/only time donor status (OR, 4.2; 95% CI, 3.9 to 4.5 compared with repeat donors). Seropositivity for human T-lymphotropic virus types I
and II, human immunodeficiency virus or hepatitis B core antigen was highly associated with HCV seropositivity (OR, 10.4; 95% CI, 9.6 to 11.4 for one vs no marker)

Qureshi et al. (1996) conducted a study on histological features of community acquired and posttransfusion hepatitis C in Karachi and found that 68 percent of community acquired hepatitis C patients had cirrhosis with chronic active hepatitis as compared to 54 percent of posttransfusion hepatitis C patients. It was also found that posttransfusion hepatitis C is more common in females because of increased likelihood of receiving transfusion for obstetric and gynecological reasons.

Arthur et al. (1997) sought the markers for hepatitis C virus infections in serum samples from 2644 blood donors in 24 of Egypt’s 26 governorates. Of the 2644 samples, 656(24.8%) were shown to contain anti-HCV immunoglobulin G antibody by Abbott second generation enzyme immunoassays(EIA).

Gosavi et al. (1997) reported from a study in Western India by using a second generation ELISA 15.9 percent blood donors, 36.4 percent multi-transfused patients, 27.8 percent renal failure patients and 26.2 percent renal transplant recipients were seropositive for anti-HCV.

Merican (1997) found a high prevalence among intravenous drug users(85.3 percent of 190), hemophiliacs(64.3 percent of 14), chronic renal failure patients on dialysis(53.9 percent of 356) and healthy asymptomatic blood donors(3 percent of 363) in Malaysia.
Mison et al. (1997) conducted a study on 34,725 first time blood donors in Brisbane to know the prevalence of anti-HCV and HCV-RNA. Out of 34,725 donors, 183 (0.55 percent) were found anti-HCV positive by a third generation ELISA, 69 (0.2 percent) were HCV-RNA positive and 0.29 percent confirmed anti-HCV positive by second and third generation RIBA.

Neogi et al. (1997) in a study from Calcutta used ELISA to test 153 serum samples obtained from different age groups and both sexes for anti-HCV. Anti-HCV was found in 13 percent of multitransfused cases and in 8.8 percent of persons with multiple needle stick injury. The highest seropositivity (20 percent) was observed among males of the 31-40 year age group. Anti-HCV was found more commonly among males (13 percent) than females (8.2 percent).

Songsivilai et al. (1997) reported in Northeast Thailand, the prevalence among 3,255 volunteer blood donors was 6.5 percent in male blood donors and 0.9 percent in female blood donors. Study also showed that prevalence increased with age, reaching a peak at 31-40 years. Approximately 80 percent of the anti-HCV positive blood donors were viremic as determined by the HCV RNA by PCR.

Ghosh (1998) reported that the seroprevalence rate among the general population in New Zealand, was low. 0.87 percent among new blood donors were ELISA anti-HCV positive, 0.17 percent were confirmed by RIBA.
Wansbrough-Jones et al. (1998) evaluated the seroprevalence of hepatitis C virus in blood donors and antenatal clinic attenders in Kumasi, Ghana. The overall seroprevalence among Ghanaians was 2.8% but there was a significantly higher prevalence in males (4.6%) than in females (1.0%).

Apichartpiyakul et al. (1999) analyzed the prevalence of antibodies against hepatitis C virus (HCV) and the distribution pattern of HCV subtypes among healthy blood donors and intravenous drug users (IVDUs) in Northern/Northeastern Thailand. The prevalence of anti-HCV antibodies was 3.2% (26/820) among blood donors in Khon Kaen. Sequence analyses of amplified fragments of the HCV genome revealed that in Khon Kaen and Chiang Rai, Thailand, HCV-3a (50-60%) was the most common HCV subtype, followed by HCV-1a, HCV-1b and subtypes of clade 6 each at 10-20%.

Leon et al. (1999) reported from his study on blood donors in Spain that type 1 was largely the more prevalent (85.5%) followed by types 3 (4.4%), 2 (4.1%), 4 (3.4%) and 5 (0.5%) and by a group of apparent mixed infections which altogether represented 2.1% of the total. Among the donors in whom the genomes were typed, infections due to the 1b subtype (78% of the 441 samples genotypes) clearly predominated.

Lo et al. (1999) conducted a survey to collect transversal data to ensure a better understanding of the hepatitis C epidemiology in Mauritania.
The authors have studied the seroprevalence rate of HCV antibodies among 349 blood donors. Data of the study showed that anti-HCV antibody was detected in 1.1% blood donors.

**Makroo et al. (1999)** conducted a study to find out the prevalence of HCV antibody in blood donors with an aim to provide safe blood for transfusion. A total of 44,086 blood samples from healthy blood donors was screened for HCV antibody by third generation ELISA assay and a prevalence of 0.53% was found in the study.

**Vardas et al. (1999)** reported the prevalence of hepatitis C virus (HCV) in Namibia as determined using a third generation enzyme-linked immunosorbent assay (ELISA) on samples of blood collected from all asymptomatic, first time blood donors between 1 February and 31 July 1997 (n=1941). The HCV seroprevalence was 0.9% (95% confidence interval (CI) : 0.5-1.5%) and no association were detected between a positive HCV serostatus and the person’s sex, region of residence, or previous hepatitis B exposure or hepatitis B carrier status, as determined by hepatitis B surface antigen (HBsAg). The only significant association in a logistic regression model was an increase in HCV positivity with increasing age (P=0.04).

**Acquaye et al. (2000)** determined the frequency of hepatitis C virus positivity in Ghananian blood donors. Blood samples from 1300 healthy
blood donors were screened for HCV antibodies by an ELISA technique. They reported 68 donors (5.2%) were positive for HCV antibodies.

Diouf et al. (2000) studied the prevalence of hepatitis C among chronic hemodialysis patients in Dakar. Among 15 chronic hemodialysis patients twelve patients (80%) were found to be HCV-antibody positive and half of them were HCV RNA positive.

Frank et al. (2000) reported that the population of Egypt has a heavy burden of liver disease mostly due to chronic infection with HCV. Overall prevalence of antibody to HCV in the general population is around 15-20%.

Ghavanini and Sabri (2000) studied 7897 voluntary blood donors in Shiraz, Islamic Republic of Iran and reported 0.59% seropositivity for antibodies to HCV.

Hatira et al. (2000) studied the hepatitis C virus antibodies by second and third generation ELISA since January 6th 1994 to December 31st 1997, in 34,130 blood donors living in ‘Sahel Tunisien’. 193 were found to be positive (0.56%) for anti-HCV.

Halim and Ajayi (2000) carried out a study to determine risk factors and seroprevalence of Hepatitis C virus antibody(anti-HCV) in blood donors in Nigeria. Sera from 260 volunteer male blood donors aged 20-54 years were screened using enzyme linked immuno absorbent assay. Anti-HCV prevalence rate was 12.3%. 56.6% of the anti-HCV positives were in the 41-
50 year age group. There was an association between anti-HCV positive and history of exposure to heterosexual partners at risk, history of sexually transmitted disease; low socio-economic status and Hepatitis B surface antigen positivity.

Inoue et al. (2000) conducted a study to describe the genetic diversity of hepatitis C virus (HCV) in a population of positive blood donors throughout Indonesia. Repeat analysis by reverse transcription polymerase chain reaction (RT-PCR) of 102 anti-HCV positive samples showed that 67 gave HCV specific positive signals by the PCR for the 5' untranslated genomic region of HCV. Further genotypic analysis on 64 HCV RNA-positive samples indicated that 57 belonged to the following individual genotypes: 1a, 1b, 2a, 2b, and 3b. The predominated HCV genotypes in this donor population were 1b(57.8%), 2a(17.2%), and 3b(10.9%).

Mujeeb et al. (2000) carried out a study to know the seroprevalence of HCV among college going first time voluntary blood donors. Three voluntary blood donors out of total 612 samples (0.5% with 95% confidence limit 0.12-1.33%) were anti-HCV positive. Seroprevalence of HCV infections among college going students is significantly low(<3.0%) than 30% seroprevalence among paid donors and 7% among family/ replacement blood donors.
Alvarez-Munoz et al. (2001) carried out a study to determine hepatitis C virus RNA (HCV-RNA) in a group of blood donors and their household contacts. Among the 44,588 donors studied, 333 (0.74%) were positive for anti-HCV. Among the 72 household members, HCV antibodies were detected in six (8.3%) members. The study shows that intrafamilial transmission of HCV may occur.

Butsashvili et al. (2001) carried out a study to determine the prevalence of Hepatitis C (HCV) virus. Out of 4970 donors 7.3% had anti-HCV (6.9% confirmed).

Kaur et al. (2001) reported HCV infections are associated with post transfusion Hepatitis. 60,780 voluntary blood donors in the state of Punjab were screened for HCV infection by enzyme immunoassay and confirmed by Immunoblot assay. HCV seroprevalence was 0.78 percent (475/60,780) by EIA.

Tamim et al. (2001) screened 5,115 blood donors in Lebanon and found that 57 were initially tested positive or doubtful for anti-HCV Antibody. Subsequent testing by two-Third generation enzyme immunoassays confirmed that of the 57 initially tested positive/doubtful, only 18 were positive for anti-HCV giving a prevalence rate of 0.4%.

Ampofo et al. (2002) investigated transfusion transmissible infections among 808 blood donors in Ghana in 1999. Antibody seroprevalence of hepatitis C virus was found to be 8.4%.
Khattak et al. (2002) collected blood donated by healthy donors from both Armed Forces and civilian population from Jan 1996 to Dec 2000. The sera were tested by Enzyme Linked Immunoassay at Armed Forces Institute of Transfusion Rawalpindi, Pakistan. Of 103858 blood donors, 4.0% (95% CI 3.91%-4.11%) were anti HCV positive.

Luksamijarulkl et al. (2002) screened 2,167 blood donors for antibodies to HCV by Enzyme Immunoassay methods. The results revealed that prevalence of anti-HCV positive among studied blood donors was 2.90%. For anti-HCV prevalence, studied variables including educational level, occupation and domicile were significant (p<0.0001, p=0.0027, and p<0.0001, respectively)

Paltanin et al. (2002) assessed epidemiological and laboratorical records of 10,090 blood donors of the blood unit in the city of Apucarana, Brazil. The results showed that of all donors 2,461(24.4%) were females, 7629 (75.6%) were males with ages ranging from 18 to 65 years old. Of 10,090 serum samples tested using ELISA, 88 were reactive to anti-HCV, a seroprevalence of 0.9% that showed no association with either age groups (p=0.197) or sex (p=0.323).

According to World Health Organization (2002), 3% of the world’s population, or approximately 170 million people are infected with Hepatitis C Virus.
Jain et al. (2003) conducted a study on 15,898 healthy voluntary blood donors. The donors were subjected to anti-HCV testing (using a commercial available third generation anti-HCV ELISA kit) and 249 were found to be reactive for anti-HCV antibody, yielding an overall prevalence of 1.57%. No significant difference was found between the HCV positivity rate of male (1.57%; 238/15,152) vs. female (1.47%; 11/746) donors, family (1.58%; 213/13,521) vs. altruistic (1.51%; 36/2377) donors and first time (1.55%; 180/11,605) vs repeat (1.61%; 69/4293) donors. The age distribution of anti-HCV reactivity showed a maximum prevalence rate of 1.8% in the age group of 20-29 years. In addition, there was a clear trend of decreasing positivity for anti-HCV with increasing age and this trend was statistically significant.

Valdivia et al. (2003) examined 15,009 blood donors to determine the predominance and some epidemiological characteristics of the HCV infection in blood donors at the Cayetano Heredia National Hospital. Out of 15,009 donors, 122 positive cases were found (0.813% prevalence). Likewise 97 cases (79.5%) were males and 25 cases (20.5%) were females. Seroprevalence predominated in the age group from 21 to 30 (36.06%), followed by the age group from 31 to 40 (27.86%).

Gupta et al. (2004) reported 1.09% anti HCV seropositivity among 44064 blood donors in Ludhiana.
Sakarya et al. (2004) reported 0.19% HCV seroprevalence in blood donors of Aydin region of Turkey.

Singh et al. (2004) reported 0.5% seropositivity for anti-HCV among 52,500 blood donors of East Delhi.

Dray et al. (2005) reported 0.3% seropositivity for antibodies to HCV among 9006 volunteer blood donors in Republic of Djibouti.

Transmission

Amarapurkar et al. (1992) from Mumbai used ELISA to detect anti-HCV in 126 patients with chronic liver disease (cirrhosis 103, cirrhosis with hepatocellular carcinoma 3, chronic active hepatitis 20) and found 21 (16.6 percent) to be positive. Of the 21 patients who tested positive, 8 (38 percent) had received blood transfusion previously.

Dasarathy et al. (1992) in a prospective study from New Delhi investigated the risk of transfusion associated hepatitis (TAH) in 215 patients who had received blood transfusions during cardiac surgery over a period of one year. Fifteen (6.9 percent) patients developed TAH during this period, of whom three had HBV induced TAH. Of the 12 NANB-TAH patients, only three (25 percent) developed anti-HCV during follow-up.

Hernandez et al. (1992) carried out a study to assess the risk to hospital personnel of acquiring an Hepatitis C Virus (HCV) infection as a result of occupational exposure to needle stick injuries. A low efficacy of
needle stick injuries in the transmission of HCV in hospital personnel may be suggested.

Domingo et al. (1997) reported the prevalence of anti-HCV in Philippines using the second generation assay to be 72 percent among 104 intravenous drug users, 11 percent among 104 multi transfused patients, 8 percent in 216 commercial sex workers, 7 percent in 101 chronic liver disease patients, 7 percent in 87 control subjects and 0.9 percent in 107 laboratory personnel.

Murphy et al. (2009) performed a case control study of 2,316 HCV-seropositive blood donors and 2,316 seronegative donors matched on age, sex, race/ethnicity, blood center and first time versus repeat donor status. Odds ratio (OR) and 95% confidence intervals (CIs) were calculated using conditional logistic regression. Questionnaire were returned by 758 (33%) HCV positive and 1,039 (45%) control subjects (P = .001). The final multivariate model included only the following independent HCV risk factors: IDU (OR=49.6; 95% CI: 20.3-121.1), blood transfusion in non-IDU (OR=10.9; 95% CI: 6.5-18.2), sex with an IDU (OR=6.3; 95% CI: 3.3-12.0), having been in jail more than 3 days (OR=2.9; 95% CI: 1.3-6.6), religious scarification (OR=2.8; 95% CI: 1.2-7.0), having been cut or stuck with a bloody object (OR=2.1; 95% CI: 1.1-4.1), pierced ears or body parts (OR=2.0; 95% CI: 1.1-3.7), and immunoglobulin injection (OR=1.6; 95% CI: 1.0-2.6).
Delage et al. (1999) carried out a study to explore risk factors predicting hepatitis C virus (HCV) infection in blood donors. In the final multivariate analysis, only 5 factors remained independently predictive of HCV infection: previous intravenous drug use (OR, 127.5; 95% CI 26.0-625.0), having lived in a prison or juvenile detention center (56.1; 11.4-275.7), previous blood transfusion (10.5; 4.7-23.2), sexual contact with an intravenous drug user (6.9; 3.1-15.2), and tattooing (5.7; 2.5-13). They concluded that most blood donors acquire infection by percutaneous exposure to contaminated blood. The study suggested a role for sexual transmission.

Katsolidou et al. (1999) reported that Haemodialysis patients are at high risk of infection by hepatitis C virus. The aim of the study was to investigate a hepatitis C virus outbreak which occurred in a haemodialysis unit, using epidemiological and molecular methods. The analysis suggested that horizontal nosocomial patient to patient transmission was the most likely explanation for the virus spread within the haemodialysis unit under study.

Elghouzzi et al. (2000) evaluated and analyzed risk factors of HCV-infected French blood donors according to HCV genotypes in order to improve the transfusion policy and safety of blood supply. The study showed that nosocomial infection may be a route of HCV spread but the main risk factor remains IVDU, particularly in Young men.
Ross et al. (2000) reported that prevention and treatment of infections with hepatitis C virus (HCV) remain a major challenge. The main source of HCV infection in developed countries was formerly transfusion of contaminated blood and blood products but is now injection drug use. In general, a potential risk factor can be established for about 90% of all cases of HCV infection. One way of contracting HCV may be transmission from infected medical personnel to susceptible patients during medical care. Provider to patient transmission of HCV is rare, and in most cases HCV-positive surgeons are the probable source. They studied an outbreak of HCV in a municipal Hospital. The findings suggest that an anesthesiology assistant contracted HCV from a chronically infected patient and subsequently transmitted the virus to five other patients.

Diagnosis (Figure 8)

Alter et al. (1989) measured antibody (anti-HCV) to Hepatitis C virus by radioimmunoassay in prospectively followed transfusion recipients and their donors. The study suggests that screening of donors for anti-HCV could prevent the majority of cases of the disease.

Donahue et al. (1992) collected blood samples and medical information from patients before and at least six months after cardiac surgery from 1985 through 1991. The stored serum samples were tested for antibodies to HCV by enzyme immunoassay, and by recombinant
Figure 8. Hepatitis C Virus (HCV) Infection-Testing Algorithm for Asymptomatic People
immunoblotting if positive. The trend toward decreasing risk with increasingly stringent screening of donors was statistically significant (P<0.001) with the volume of blood transfused, but not with the use of particular blood components. The incidence of post-transfusion hepatitis C has decreased markedly since the implementation of donor screening for surrogate markers and antibodies to HCV. The current risk of post transfusion hepatitis was found to be about 3 per 10,000 units transfused.

Mitsui et al. (1992) documented Hepatitis C virus infections in medical personnel after needlestick accidents, generally by detection of seroconversion to a hepatitis C virus nonstructural region antigen, c100-3 (a marker of infection). It was found in the study that the risk of hepatitis C virus transmission from a single needlestick accident with hepatitis C virus RNA-positive blood was 10%. Donor blood with antibody to an hepatitis C virus core-derived peptide with enzyme-linked immunosorbent assay optical densities greater than 2.0 carried a significant risk of transmitting hepatitis C virus to needlestick victims.

Chung et al. (1993) reported that antibody to hepatitis C virus should be screened in all blood donors to minimize the risk of post transfusion C.

Vrielink et al. (1997) compared the performance of three generations of anti-HCV enzyme-linked immunosorbent assay (ELISA) (ELISA- 1, -2, -3) in routine blood donor screening (99,394 donations were tested with
ELISA –1, 167,999 donations with ELISA –2, and 262,090 donations with ELISA –3). The study shows that donor population that previously tested negative with ELISA –2, but it did detect HCV antibodies earlier in some patients with acute HCV infection. ELISA –2 and –3 were significantly more sensitive than second – and third generation recombinant immunoblot assays.

Pawlotsky et al. (1998) carried out a study to determine a cost-effective strategy for the diagnosis of hepatitis C virus, infection in clinical laboratories. Results of the study showed that confirmatory assay do not add any information regarding the presence of anti-HCV antibodies in the samples found positive in ELISA and they are little contributive in resolving weakly positive or discrepant results of screening assays, because they can be positive, indeterminate or negative, with or without HCV replication. The study suggests that one single ELISA determination is necessary for diagnosis of HCV infection in clinical laboratories, and confirmation of positive or weakly positive ELISAs with immunoblot based confirmatory assays is no longer needed.

Grant et al. (2002) reported that detection of HCV during the window phase of infection before seroconversion is important in blood screening. HCV RNA levels were measured before seroconversion and compared with HCV core antigen and anti-HCV detection. A wide range of HCV RNA levels can be detected during the seronegative window phase of
HCV infection. HCV core antigen can be used to detect HCV infection during the window phase of infection.

**Kiely et al. (2002)** compared the sensitivity of two commercially available anti-HCV immunoblot assays (HCV Western blot(Wellcozyme) and RIBA 3.0 SIA [RIBA-3, Chiron]) in a voluntary blood donor population. Western Blot and RIBA-3 showed similar sensitivity to the core and NS5 proteins. However RIBA-3 showed greater sensitivity to both NS3 and NS4 compared to the western blot. The reduced sensitivity of the western blot to the NS3 and NS4 proteins was observed with HCV type 3 samples.

**Muerhoff et al. (2002)** reported that currently, the detection of HCV infection in blood donors relies on the ability of immunoassay to detect circulating HCV antibodies. However, a significant delay exists between the time of infection and the development of antibodies. This delay (window period) can last up to 70 days. The introduction of Nucleic Acid Testing (NAT) for the detection of HCV RNA has reduced this window period dramatically. However, NAT is labor intensive, prone to contamination and expensive as compared with standard serologic tests. The data indicates that the automated microparticle based chemiluminescent HCV core antigen assay can reduce the window period for detection of potentially infected blood donors by 32.7 days and it represents a viable alternative to HCV RNA testing.
Nubling et al. (2002) reported that various countries have introduced HCV NAT to exclude infectious donations collected during the preseroconversion window phase (PWP). For the same purpose, an ELISA has also been developed to detect HCV core antigen (cAg). A majority of HCV RNA-positive samples were also cAg-positive during the PWP.

**Stability of HCV**

Grant et al. (2000) studied the stability of hepatitis C virus (HCV) RNA during transport and storage of blood samples from donors, prior to screening for HCV by nucleic acid amplification technology. Various blood and plasma sample types were stored for up to 120h at different temperatures and the HCV RNA level was observed after 72h storage of whole blood at 4°C in EDTA tubes (Greiner) and plasma preparation tubes (PPT; Becton Dickinson), while insignificant declines of $0.2\log_{10}$ and $0.25\log_{10}$ occurred at 25°C after 72h in the EDTA tubes and PPT tubes respectively. When whole blood was stored with mixed anticoagulants CPDA-1 and EDTA for up to 12h, no decline in HCV RNA level was observed at 4°C and 25°C, while a significant decline of $0.37\log_{10}$ occurred at 37°C after 120h. The temperature during transportation was investigated with 120h period at 25°C and 37°C before storage at 4°C for 108h. Neither temperature resulted in any loss of HCV RNA in comparison with 120h of storage at 4°C. Whole blood anticoagulant with EDTA or CPDA-1/EDTA may be stored at up to 25°C (room temperature) for up to 5 days without any significant loss in plasma HCV RNA level.
Treatment Of Hepatitis C (Figure 9)

**Hoofnagle et al. (1986)** carried out a study to treat 10 patients who had chronic non-A, non-B hepatitis with recombinant human alpha interferon in varying doses (0.5-5 million units) daily, every other day, as three times weekly for up to 12 months. They reported that prolonged treatment was associated with a sustained improvement in aminotransferase levels; in these cases, biopsy specimens obtained after one year of therapy showed marked improvement in hepatic histology, even though low doses of alpha interferon had been used.

**Nishiguchi et al. (1995)** showed that IFN-alpha improved liver function in chronic active hepatitis C with cirrhosis, and its use was associated with a decreased incidence of hepatocellular carcinoma.

The initial therapy with interferon alfa (or equivalent) should be 3 million units three times per week for 12 months. Patients not responding to therapy after 3 months should not receive further treatment with interferon alone, but should be considered for combination therapy of interferon and ribavirin or for enrollment in investigational studies. **NIH (1997)**

**Yamamoto et al. (1997)** studied the correlation of interferon responses or other clinical features with Internal Ribosome Entry Site (IRES) sequence variability. Sequence variability of the IRES has no influence on interferon efficacy or serum HCV-RNA concentrations in
Figure 9. The Evolution Efficacy With Interferon Based Therapy Over the Last 10 Years
patients with chronic HCV-1b infection. The results indicate that diverse clinical pictures among patients with a given genotype of HCV could not be explained by the sequence variability seen in the IRES that is thought to have a key role in both HCV-RNA replication and translation. Therefore, variability in other parts of the HCV genome appears to be important for the different clinical pictures. There is significant association between mutations in the NS5A region and responses to interferon.

**Mc Hutchison et al. (1998)** assigned 912 patients with chronic hepatitis C to receive standard dose interferon alfa-2b alone or in combination with ribavirin (1000 or 1200 mg orally per day, depending on body weight) for 24 or 48 weeks and reported that among patients with HCV genotype 1 infection, the best response occurred in those who were treated with combination therapy for either 24 weeks (44%) or 48 weeks (41%).

**Neumann et al. (1998)** reported that the major initial effect of IFN is to block virion production or release, with blocking efficacies of 81, 95 and 96%, for daily doses of 5, 10 and 15 million international units, respectively. They showed that infection with hepatitis C is highly dynamic and that early monitoring of viral load can help guide therapy.

**Sangiovanni et al. (1998)** reported that 6 months’ treatment with IFN-alpha-2a did not eradicate HCV RNA from serum in carriers with persistently normal ALT levels but caused ALT flare-ups in two thirds of
them. Until more is known about the natural history of HCV RNA carriers with normal ALT levels, these patients should not be treated with IFN.

Gruner et al. (2000) reported that the number of IFN-gamma-producing HCV-specific CD8+ T cells during the first 6 months after onset of disease is associated with eradication of the HCV infection.

Heathcote et al. (2000) reported that in patients with chronic hepatitis C but without cirrhosis, once weekly administration of interferon modified by the attachment of a 40 kd branched chain polyethylene glycol moiety (peginterferon alfa-2a) is more efficacious than a regimen of unmodified interferon.

Nelson et al. (2000) observed that IL-10 therapy was associated with changes in serological markers, suggesting a reduction of immune response and fibrogenesis.

Fried et al. (2002) conducted a study on treatment with peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection and showed that in patients with chronic hepatitis C, once-weekly peginterferon alfa-2a plus ribavirin was tolerated as well as interferon alfa-2b plus ribavirin and produced significant improvements in the rate of sustained virologic response, as compared with interferon alfa-2b plus ribavirin or peginterferon alfa-2a alone.
Legrand-Abravanel et al. (2004) reported that HCV genotype 5 might have good intrinsic sensitivity to combination therapy with interferon-\(\alpha\) plus ribavirin.

Tanabe et al. (2004) suggested that the direct effects of ribavirin on the genetic stability of the HCV subgenome and its synergistic action combined, with IFN-\(\alpha\), may explain the improved clinical responses to combination therapy.