HEPATITIS C VIRUS

1. Exposure Data

1.1 Structure and biology of hepatitis C virus (HCV)

1.1.1 *Structure of the virus*

The etiological agent of most cases of post-transfusion hepatitis and a variable proportion of sporadic non-A, non-B hepatitis was discovered in 1989, by recombinant cDNA immunoscreening of serum from a chimpanzee chronically infected with a contaminated human factor VIII concentrate (Choo *et al.*, 1989). The agent was termed hepatitis C virus (HCV). It is a positive-strand RNA virus distantly related to the pestiviruses and flaviviruses on the basis of similar biophysical characteristics, genome organization, hydrophobicity plots and consensus sequences (Miller & Purcell, 1990; Han *et al.*, 1991; Koonin, 1991).

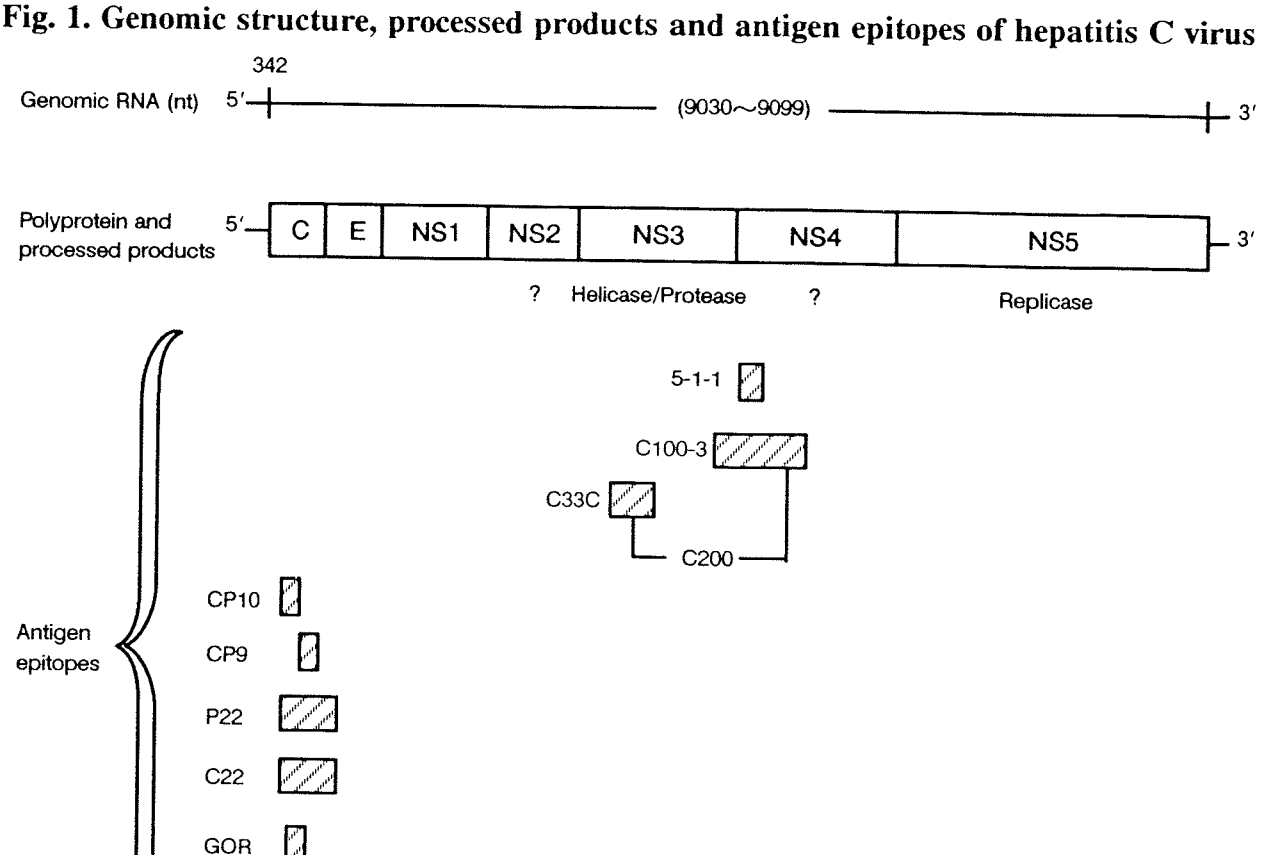
1.1.2 *Structure of HCV genome and gene products*

The genomic organization and characterization of HCV have been described (Choo *et al.*, 1991; Han *et al.*, 1991; Cha *et al.*, 1992). The viral genome is a positive-strand RNA molecule about 9.4 kilobases long (Fig. 1), which is translated into a viral polyprotein. The prototype HCV nucleotide sequence is called HCV-1 (Choo *et al.*, 1991). The single large open reading frame encodes a polyprotein precursor of about 3010 amino acids, which contains co-linearly structural and nonstructural proteins. Putative boundaries are assigned that separate the 5′ untranslated region, the core protein, the glycoprotein envelope 1 (E1), the nonstructural protein 1/envelope 2 (NS1/E2), the nonstructural proteins 2–5 (NS2, NS3, NS4 and NS5) and the 3′ untranslated region.

1.1.3 *Replication and gene expression of HCV*

HCV RNA is transcribed into minus-strand RNA, the putative replication intermediate. HCV does not appear to produce DNA replicative intermediates, and integrated viral sequences have not been found in the host genome (Choo *et al.*, 1989). Viral proteins or viral particles have not been identified in serum from HCV-infected individuals; however, viral antigens have recently been detected in infected hepatocytes by immunohistochemical analysis (Hiramatsu *et al.*, 1992; Krawczynski *et al.*, 1992). Apart from a study using a human T-cell line (Shimizu, Y.K. *et al.*, 1992), infection of cells with HCV *in vitro* has not been reported.

Fig. 1. Genomic structure, processed products and antigen epitopes of hepatitis C virus



nt, nucleotide; C, core protein; E, envelope; NS, nonstructural protein
From Greenwood & Whittle (1981); Alter, H.J. *et al.* (1989); Kuo *et al.* (1989); McFarlane *et al.* (1990); Mishiro *et al.* (1990); Okamoto *et al.* (1990a); Chiba *et al.* (1991); Houghton *et al.* (1991); Suzuki *et al.* (1991); Watanabe *et al.* (1991a,b); Weiner *et al.* (1991a); Bresters *et al.* (1992); Claeys *et al.* (1992); Kotwal *et al.* (1992a); Matsuura *et al.* (1992); van der Poel *et al.* (1992); Watanabe *et al.* (1993)

It has been possible to identify viral proteins by transcription of RNA from cloned HCV cDNA as well as by transfection of cell lines (Harada *et al.*, 1991; Kumar *et al.*, 1992) *in vitro*, followed by translation of the RNAs *in vitro*. HCV core and envelope proteins, encoded by the NS1/E2 region of the viral genome, have been expressed *in vitro* in *Escherichia coli* (Mita *et al.*, 1992), in insect cells (Matsuura *et al.*, 1992) and in mammalian cells (Matsuura *et al.*, 1992; Spaete *et al.*, 1992). The E2 protein appears to have an amino-terminal hypervariable region that may be the target of immune selection of HCV variants and may be found sequentially in infected individuals (Weiner *et al.*, 1992). The full-length protein NS1/E2 appears to be cell-associated and is not secreted; in contrast, C-terminal truncated proteins were detected extracellularly and may be relevant targets for the host immune response and therefore potential subunit vaccine candidates (Spaete *et al.*, 1992). In the natural course of HCV infection, however, antibodies to the NS1/E2 protein are detected infrequently and do not serve as evidence of viral clearance (Matsuura *et al.*, 1992; Mita *et al.*, 1992).

1.1.4 *HCV animal models*

HCV has been detected in humans and has been successfully transmitted to chimpanzees. At present, the chimpanzee is the only established animal model for non-A, non-B hepatitis (Alter *et al.*, 1978; Tabor *et al.*, 1978; Bradley *et al.*, 1979; Wyke *et al.*, 1979; Yoshizawa *et al.*, 1980) and HCV infection specifically (Shimizu *et al.*, 1990). The natural course of HCV infection has been studied in this animal model (Bradley *et al.* 1990; Abe *et al.*, 1992; Beach *et al.*, 1992; Farci *et al.*, 1992a; Hilfenhaus *et al.*, 1992; Shindo *et al.*, 1992a). Most importantly, studies in champanzees reveal a lack of protective immunity against reinfection with HCV (Farci *et al.*, 1992b; Prince *et al.*, 1992). In contrast to hepatitis B virus (HBV), no naturally occurring HCV-related animal virus has been identified.

1.1.5 *Genotypes of HCV*

After the initial discovery of HCV (Choo *et al.*, 1989), viral isolates from different parts of the world were sequenced, and a huge amount of information on HCV diversity has been published. Complete HCV cDNA sequences have been established for isolates from the USA, including the initial clone HCV-1 (Choo *et al.*, 1991) and the HCV-H virus (Inchauspé *et al.*, 1991), and for isolates from Japan, including HCV-J (Kato *et al.*, 1990), HCV-BK (Takamizawa *et al.*, 1991), HCV-J4 (Okamoto *et al.*, 1990a, 1992a), HCV-J6 (Okamoto *et al.*, 1991) and HCV-J8 (Okamoto, 1992; Okamoto *et al.*, 1992a). Partial HCV cDNA sequences are known for isolates from the USA (Weiner *et al.*, 1991), Japan (Enomoto *et al.*, 1990; Maéno *et al.*, 1990; Okamoto *et al.*, 1990a; Takeuchi *et al.*, 1990a; Hijikata *et al.*, 1991; Tsukiyama-Kohara *et al.*, 1991), Thailand (Mori *et al.*, 1992), China (Chen *et al.*, 1992; Liu *et al.*, 1992; Wang Y. *et al.*, 1992), France (Kremsdorf *et al.* 1991; Li *et al.*, 1991), Germany (Fuchs *et al.*, 1991) and Scotland (Chan, S.-W. *et al.*, 1992).

Comparison of the sequence of the original HCV-1 isolate from the USA (Choo *et al.*, 1989) with a Japanese isolate, HCV-J (Kato *et al.*, 1990), revealed that these HCVs differ both in nucleotide and polypeptide sequence (Kubo *et al.*, 1989; Takeuchi *et al.*, 1990b; Choo *et al.*, 1991). On the basis of nucleotide sequence homology, several genotypes have been identified throughout the world (Enomoto *et al.*, 1990; Houghton *et al.*, 1991; Cha *et al.*, 1992; Chan, S.-W. *et al.*, 1992; Okamoto *et al.*, 1992a,b). On the basis of nucleotide sequence homology of whole sequenced HCV isolates, they were classified into type I (1a), type II (1b), type III (2a) and type IV (2b). Provisionally, type V (3a) and type VI (3b) isolates were reported on the basis of data on partially sequenced genomes.

Apart from the geographic distribution of HCV genotypes mentioned above, recent evidence suggests that HCV exists in infected individuals as different but related genomes, known as quasispecies (Martell *et al.*, 1992; Murakawa *et al.*, 1992; Tanaka, T. *et al.*, 1992; Weiner *et al.*, 1992). Researchers have proposed many classification schemes based primarily on nucleotide sequence homology using different regions of the genome. There is no universally agreed classification.

1.1.6 *Host range and target cells of HCV infection*

The host range of HCV is very narrow, as HCV infects only humans and chimpanzees. The molecular basis of this narrow host range is not known.

In permissive hosts, viral antigens and nucleic acids are found primarily in serum and liver cells. In infected liver tissues, HCV antigens have been detected by immunohistochemical analysis (Hiramatsu *et al.*, 1992; Krawczynski *et al.*, 1992), and RNA has been found in liver and serum by molecular techniques (Fong *et al.*, 1991; Akyol *et al.*, 1992; Bresters *et al.*, 1992; Diamantis *et al.*, 1992; Hosoda *et al.*, 1992; Lamas *et al.*, 1992; Negro *et al.*, 1992; Takehara *et al.*, 1992). HCV RNA has also been detected in peripheral blood mononuclear cells (Qian *et al.*, 1992; Hsieh *et al.*, 1992; Wang, J.-T. *et al.*, 1992; Zignego *et al.*, 1992). Experimental evidence was obtained recently for in-vitro infection and replication of HCV in a human T-cell line (Shimizu, Y.K. *et al.*, 1992).

The biological significance of HCV in cells other than hepatocytes remains largely undefined, however. Blood mononuclear cells may play a critical role in reactivation episodes during chronic HCV infection, after interferon treatment of chronic hepatitis C (Qian *et al.*, 1992) and in reinfection after liver transplantation (Read *et al.*, 1991; Ferrell *et al.*, 1992a; Belli *et al.*, 1993).

1.2 Methods of detection

The detection of infection is based upon assays for viral antibodies and viral nucleic acids. In contrast to HBV infection, no assay system is yet available commercially for detection of HCV antigens in serum or plasma, although they can be detected in serum by research techniques, such as enzyme-linked immunosorbent assay (ELISA) (Takahashi *et al.*, 1992).

1.2.1 *In serum and plasma*

Tests for anti-HCV first became available in 1989. These are known as first-generation assays and had limited sensitivity and specificity; they have been superseded by improved second-generation assays. Neither test distinguishes between current and past HCV infection.

(*a*) *The first-generation anti-HCV assay*

In this assay, C100-3 antigen, a recombinant antigen derived from the NS3–NS4 region of the HCV genome (Fig. 1), is used to capture anti-HCV. The labelled anti-HCV is then detected by a second labelled antibody to human immunoglobulin (Kuo *et al.*, 1989). This assay is now available commercially in the ELISA format. In the USA, anti-C100-3 was used to detect anti-HCV in radioimmunoassays in about 90% of blood units implicated in post-transfusion hepatitis (Alter, H.J. *et al.*, 1989).

Screening for anti-C100-3 before blood transfusion reduced the number of cases of post-transfusion non-A, non-B hepatitis in Japan by 60–80% (Japanese Red Cross Non-A, Non-B Hepatitis Research Group, 1991). This test also detected anti-HCV in about 60% of HCV RNA-positive blood donors (Watanabe *et al.*, 1993).

The specificity of the assay is reduced by freezing and thawing serum or plasma and by the presence of high immunoglobulin levels (McFarlane *et al.*, 1990). The latter aspect is particularly important, since in many etiological forms of chronic liver disease immunoglobulin levels are typically elevated, and in individuals living in tropical areas immuno-

globulin levels can be very high owing to chronic parasitic infections (Greenwood & Whittle, 1981).

(*b*) *The second-generation anti-HCV assays*

In second-generation anti-HCV assays, a recombinant antigen of the non-structural NS3 region, named C33c, and a recombinant antigen of the nucleocapsid (core) region, named C22, were added to the previously used C100-3 antigen for a second-generation ELISA. In another assay, C200 antigen expressed as one polypeptide comprising C100-3 and C33c is used as antigen together with C22 (Bresters *et al.*, 1992; van der Poel *et al.*, 1992). Agglutination tests have also been developed in which gelatin particles coated with second-generation antigens (particle agglutination) and fixed erythrocytes coated with second-generation antigen (passive haemagglutination) have been used to measure anti-HCV. More than 98% of RNA-positive serum samples are detected in second-generation assays for anti-HCV as compared with about 60% in first-generation assays (Watanabe *et al.*, 1993).

Several other systems using other antigens, e.g. NS5, have been described. The second-generation assays are more specific and sensitive than the first-generation assays and to a large degree overcome the limitations mentioned above.

(*c*) *Confirmatory tests*

Confirmatory recombinant immunoblot assays (RIBA) and neutralization assays were developed using different viral antigens. The inclusion of additional antigens and a format different from the ELISA improves the specificity of the test. Confirmatory tests give positive results in more than 90% of patients with chronic liver disease or post-transfusion hepatitis tested by ELISA (Suzuki *et al.*, 1991; Watanabe *et al.*, 1991a).

(*d*) *Other anti-HCV assays*

Assays have also been based on core and core-related antigens (see Fig. 1), including CP9 (Okamoto *et al.*, 1990b), CP10 (Okamoto *et al.*, 1992c), P22 (Chiba *et al.*, 1991), HCV core (Claeys *et al.*, 1992), HCV-SP (synthetic polypeptide) (Kotwal *et al.*, 1992a), N14 (Watanabe *et al.*, 1993) and GOR (Mishiro *et al.*, 1990; Watanabe *et al.*, 1991b). The relevance of these assays to the natural history of the disease remains to be established.

(*e*) *HCV RNA*

HCV infection can also be assessed by detecting HCV RNA by reverse transcription (RT) and the polymerase chain reaction (PCR), which is highly sensitive and has been used for early diagnosis: Quantitative PCR can be used to detect 5–30 molecules of synthetic HCV RNA (Hagiwara *et al.*, 1993). The detection limit of PCR is at present about 10 chimpanzee infectious doses per millilitre of serum (Okamoto *et al.*, 1990c).

Well-controlled procedures for handling samples, extraction and purification of nucleic acids, avoidance of laboratory contamination and use of appropriate negative and positive controls are essential prerequisites for the PCR assay. Selection of primers from the highly conserved 5′ non-coding region is also important for sensitivity and has allowed identification of a broad range of genotypes (Okamoto *et al.*, 1990c).

Testing by PCR has become the 'gold standard' for some workers. The results of these tests correlate well with the risk for transmitting post-transfusion hepatitis, with those of

second-generation anti-HCV assays and with liver histology and are useful in monitoring the response of patients to interferon therapy. The test suffers from the risk for contamination, however, and reproducibility between laboratories has been poor (Zaaijer *et al.*, 1993).

1.2.2 *In liver tissues*

(*a*) *HCV antigen*

HCV antigen can be detected immunohistochemically using fluorescein isothiocyanate-labelled immunoglobulin G fractions from chimpanzee and human sera that are strongly reactive with recombinant structural and non-structural proteins of HCV. In one study, the antigen was localized in the cytoplasm of hepatocytes in all nine chimpanzees with acute hepatitis C, in 5/10 chimpanzees with chronic HCV infection and in 11/12 patients with chronic hepatitis C. Direct immunomorphological evidence for the presence of HCV antigen deposits in hepatocytes using fluorescein isothiocyanate-labelled polyclonal anti-HCV antigen probe was established in absorption experiments using recombinant HCV non-structural proteins. The putative HCV NS3 protein was the most readily detected component of HCV in liver cells (Krawczynski *et al.*, 1992).

(*b*) *HCV RNA*

HCV RNA can be detected in liver biopsy samples from patients with chronic hepatitis C by RT–PCR and confirmed by Southern blotting. Shieh *et al.* (1991) used primers from both NS3 and core regions and detected the NS3 region more frequently than the core region. HCV RNA was localized by in-situ hybridization in the cytoplasm of hepatocytes in liver biopsy samples obtained from patients with chronic non-A, non-B hepatitis who were sero-positive for anti-HCV.

The presence of minus-strand HCV RNA was tested in blood and liver specimens from patients with HCV infection, but it was detected only in the liver. These results suggest that HCV replicates predominantly in liver cells. The detection of minus-strand HCV RNA should be useful for determining HCV replication in tissues other than liver (Takehara *et al.*, 1992).

1.2.3 *Interpretation of serological markers of HCV infection*

Patients infected with HCV may or may not develop clinical and biochemical evidence of acute hepatitis. First-generation assays may give positive results at the time of acute hepatitis or not for months after acute infection, so repeat testing up to 12 months after onset of disease is necessary before HCV infection can be ruled out as a cause of non-A, non-B hepatitis. Even then, given the limited sensitivity and specificity of first-generation assays, the diagnosis cannot be made with certainty. Second-generation assays usually give positive results at the onset of clinical disease, but repeat testing may be necessary even with these tests. After acute infection, approximately 50% of patients become asymptomatic and have normal transaminase levels (Alter *et al.*, 1992); however, anti-HCV remains and RNA is found by PCR in the majority of cases, suggesting persistent infection.

About 50% of patients with clinical evidence of acute HCV infection develop persistent or fluctuating increases in the level of alanine aminotransferase, and most of them have a

histological picture of chronic active hepatitis in liver biopsy samples, which may progress to cirrhosis. PCR shows that they also retain anti-HCV and HCV RNA. Thus, the results of tests for both anti-HCV and HCV RNA are usually positive in both individuals with active and those with quiescent HCV infection. Current evidence suggests that few patients resolve HCV infection spontaneously (Alter *et al.*, 1992).

Existing immunoglobulin M–anti-HCV tests, although not commercially available, may prove useful in differentiating acute HCV from exacerbations of chronic disease.

1.3 Epidemiology of infection

Specific tests for hepatitis C became available in 1989 (Kuo *et al.*, 1989), although the existence of a virus distinct from viruses A and B that causes post-transfusion hepatitis had been proposed for years previously (Prince *et al.*, 1974; Anon., 1975). Studies in the USA showed that sporadic cases occurred in addition to those associated with blood transfusion (Alter *et al.*, 1982). The availability of specific tests has begun to clarify the epidemiology. A significant number of false-positive results was obtained using early tests with an ELISA to the C100-3 antigen, particularly in the populations of tropical countries; use of the second-generation tests and confirmation by RIBA has provided more reliable estimates of the prevalence of infection. Table 1 shows the prevalences of specific antibody in various populations and the assays used.

Table 1. Community-based studies of seroprevalence to HCV markers

Region	Assay[a]	Age group (years)	No. of people	% with Ab	Comments	Reference
Cameroon	RIBA	16–70	315	9.8	Rate increased with age; excess in women over men	Mencarini *et al.* (1991)
Swaziland	RIBA	16–50	194	1.5		Aceti *et al.* (1992)
Italy	1st-gen. ELISA	20– ≥ 61	812	2.9	Rate increased with age; excess in men over women	Albano *et al.* (1992)
Italy	RIBA-2	30–69	1484	0.87	Higher prevalence in those 40–59 years old	Rapicetta *et al.* (1992)
Spain	1st-gen. ELISA	6 mo.–75	497	0.61	Excess in men over women	Dal-Ré *et al.* (1991)
Peru	RIBA	14–80	2111 (males)	0		Hyams *et al.* (1992)
Yemen	RIBA	3–80	348	2.6	Rate increased with age	Scott *et al.* (1992)
Hong Kong	1st-gen. ELISA	0– > 60	382	0.5		Chan, G.C.B. *et al.* (1992)
Japan	1st-gen. ELISA	> 40	1009	2.3	Rate increased with age; excess in men over women	Ito *et al.* (1991)
USA	RIBA	≥ 15	2523	18.0		Kelen *et al.* (1992)

[a]ELISA, enzyme-linked immunosorbent assay; RIBA, recombinant immunoblot assay: sera were initially screened using first- and second-generation ELISA, and reactive sera were further verified with a second-generation immunoblot assay; RIBA-2, second-generation RIBA, with C22, 5.1.1, C100-3 and C33 antigens (sometimes called RIBA 4)

Screening of blood donors has also provided information on prevalence, although the exclusion of high-risk groups and those with a history of hepatitis makes these populations less representative. The results of a sample of donor surveys are shown in Table 2. Surveys have also been carried out of pregnant women (Table 3).

Table 2. Prevalence of HCV antibodies among blood donors in various regions

Country or region	Assay[a]	Age group (years)	No. of people	% with Ab	Reference
Niger	2nd-gen.	mean, 30	1068 men	0.56	Develoux *et al.* (1992) (abstract)
United Kingdom	RIBA-2	NR	31 936	0.08	Goodrick *et al.* (1992)
Germany	1st-gen.	18–65	116 700	0.72	Caspari *et al.* (1991)
Saudi Arabia	RIBA (5-1-1, C100-3)	NR	4580 Saudis 1694 Middle East 1824 Far East 2548 European/ American	0.33 1.42 0.27 0.27	Bernvil *et al.* (1991)
Kuwait	1st-gen.	NR	505	3.0	Al-Nakib *et al.* (1992)
Thailand	1st-gen.	10–70	390	2.6	Boonmar *et al.* (1990)
Hong Kong	1st-gen.	NR	4291	1.24	Lin *et al.* (1992)
China	RIBA-2	18–50	503	1.6	Zhang *et al.* (1992)
Japan	1st-gen.	15– > 60	2970	1.14	Watanabe *et al.* (1990)
Australia	RIBA-2 or -4	20–60	94 970	0.31	Archer *et al.* (1992)

NR, not reported

[a]RIBA-2, second-generation recombinant immunoblot assay using 5-1-1, C100-3, C33c and C22-3 antigens: a positive reaction is reactivity against any two of the four antigens; 1st-gen., first generation enzyme-linked immunosorbent assay; RIBA-2 or -4, second-generation RIBA with 5-1-1, C100-3, C33c and C22-3 antigens or only the first two: a positive reaction is reactivity against either the two antigens or the four antigens.

All three survey populations show the same pattern of infection, with rates of 1% or lower in Europe and North America when the RIBA is used, and rates of 1–3% in the Middle East and parts of Asia; only in Central Africa are higher rates seen. In all of these surveys, rates increased with age, particularly after the age of 30. The sex ratio varied from a 2:1 excess in men to an excess in women. In the study of blood donors in Australia, there was a peak prevalence in younger adults (30–34 years in each sex) (Archer *et al.*, 1992).

Specific studies of prevalence have also been carried out in groups considered to be at increased risk. These can be divided into those in which parenteral transmission is considered a risk and those in which risk is considered to increase owing to other behaviour patterns.

1.3.1 *Parenteral exposure*

(*a*) *Occupation*

In Japan, all reported 'needle-stick' injuries in staff at one hospital were studied over the period 1981–89 (Kiyosawa *et al.*, 1991). A total of 110 employees received such injuries while

Table 3. Seroprevalence of HCV antibodies in pregnant women in various regions

Country or region	Assay[a]	Age group (years)	No. of people	% with Ab	Reference
Niger	2nd-gen.	mean, 24.3	355	0	Develoux *et al.* (1992) (abstract)
France	RIBA-2	< 20–> 40	1089	North African, 1.9 Black African, 4.8 European, 0 Asian, 1.8	Aussel *et al.* (1991)
France	RIBA-2	17–45	2367	French, 0.99 African, 1.06	Roudot-Thoraval *et al.* (1992)
Spain	1st-gen.	Not reported	241	1.2	Esteban *et al.* (1989)
USA	RIBA-2	13–43	1005	1.8	van Bohman *et al.* (1992)
Thailand	1st-gen.	18–35	212	2.8	Boonmar *et al.* (1990)
Taiwan	RIBA	23–36	944	0.63	Lin *et al.* (1991)

RIBA-2, second-generation recombinant immunoblot assay; 1st-gen., first-generation enzyme-linked immunosorbent assay

treating HCV-seropositive individuals; four developed acute hepatitis, three seroconverted to anti-HCV, and the remainder did not seroconvert to anti-HCV. A study of 456 New York (USA) dentists in 1985–87 (Klein *et al.*, 1991) demonstrated prevalences of anti-HCV (by ELISA confirmed with RIBA) of 1.75% in male and 1.6% in female dentists and 0.14% among blood donors with at least one year of post-graduate education. The only significant association with HCV seropositivity in this study was with oral surgery; the HCV-seropositive dentists reported having treated more AIDS patients, homosexual men, intravenous drug users and haemophiliacs than those who were seronegative. In contrast, 94 dentists in south Wales (United Kingdom) were all found to be seronegative for anti-HCV (Herbert *et al.*, 1992). In Germany, the prevalence of antibodies to HCV (analysed by RIBA) was 0.58% among 1033 hospital employees and 0.24% among blood donor controls (Jochen, 1992). A study of 945 hospital workers in southern Italy found 4.8% to be seropositive, with a seroprevalence of 1.1% of 3575 blood donor controls (De Luca *et al.*, 1991); 576 factory workers from the same area had a 10% seroprevalence (De Luca *et al.*, 1992). In a haemodialysis unit in Italy, 2.5% of staff members were seropositive for antibodies to HCV (Maggi & Petrarulo, 1992).

(*b*) *Bleeding disorders*

The prevalence of HCV antibody in people with haemophilia A or B or von Willebrand's disease, who receive clotting factors, is shown in Table 4. The first-generation assays appeared to be less sensitive than the second-generation assays.

Table 4. Prevalence of antibodies to HCV in people with blood clotting disorders

Country or region	No. of people	Bleeding disorder	Assay[a]	% with Ab	Reference
Spain	97	Haemophilia	1st-gen. RIA	63.9	Esteban *et al.* (1989)
Australia	176	165 with haemophilia A, 5 with haemophilia B, 6 with von Willebrand's disease	1st-gen. ELISA	75.6	Fairley *et al.* (1990)
Germany	28	Haemophilia	ELISA	85.7	Abb (1991)
Sweden	141	112 with haemophilia A, 29 with haemophilia B	1st-gen. ELISA	86.5	Widell *et al.* (1991)
USA	131	117 with haemophilia A, 12 with haemophilia B, 1 asymptomatic haemophila carrier, 1 with von Willebrand's disease	1st-gen. ELISA	76.3	Brettler *et al.* (1990)
Scotland	78	66 with haemophilia A, 19 with haemophilia B	RIBA-2	96.2	Watson *et al.* (1992)
France	42	Haemophilia	EIA-2	100	Laurian *et al.* (1992)
Australia	392	331 with haemophilia A, 40 with haemophila B, 21 with von Willebrand's disease	1st-gen. ELISA	73.0	Leslie *et al.* (1992)

[a]1st-gen. RIA, first-generation radioimmunoassay; 1st-gen. ELISA, first-generation enzyme-linked immunosorbent assay; RIBA-2, second-generation recombinant immunoblot assay; EIA-2, second-generation enzyme immunoassay

(c) *Renal patients*

A number of studies have been carried out in patients undergoing haemodialysis for renal failure or who have received renal transplants (Table 5). These studies show a relationship between HCV seropositivity and previous blood transfusion and duration of haemodialysis. In peritoneally dialysed patients, previous haemodialysis was a significant risk factor for seropositivity; the first-generation assays had a significant rate of false-negativity.

Kidney transplant patients in France had a seroprevalence of antibodies to HCV of 23.6% (Pol *et al.*, 1992). Of 27 patients followed prospectively, 10 (37%) were already seropositive at the time of transplantation and remained anti-HCV seropositive during follow-up, 11 (41%) patients developed antibody at an average of 95 months after renal transplantation, and six initially seropositive patients (22.2%) lost antibody at an average of 111 months after transplantation. In a similar study in Spain, 32 (48%) of 67 patients were seropositive at the time of transplantation, nine of the 32 (28%) lost antibody after transplantation and five of the remaining 35 seronegative patients (14%) became seropositive (Ponz *et al.*, 1991).

(d) *Intravenous drug users*

Studies of seroprevalence for antibodies to HCV among intravenous drug users are summarized in Table 6. Rates of infection are high in all geographical areas, and, when it was

Table 5. Prevalence of antibodies to HCV in patients on renal dialysis

Country or region	Treatment	Assay[a]	No. of people	Prevalence (%)	Reference
New Zealand	Peritoneal dialysis	EIA-2	35	8.6	Blackmore *et al.* (1992)
	Haemodialysis		53	1.9	
	Transplantation		155	4.5	
Germany	Haemodialysis	ELISA-2	498	23.1	Schlipköter *et al.* (1992)
Italy	Peritoneal dialysis	RIBA	64	4.8	Brugnano *et al.* (1992)
	Haemodialysis		205	13.3	
Italy	Haemodialysis	ELISA C100	177	10.2	Fabrizi *et al.* (1992)
Italy	Haemodialysis	RIBA	146	21.9	Maggi & Petrarulo (1992)
Italy	Haemodialysis	ELISA-2	185	38.0	Mosconi *et al.* (1992)
Italy	Haemodialysis	RIBA	318	25.5 (mean of 3)	Vandelli *et al.* (1992)
Saudi Arabia	Haemodialysis	RIBA	66	45.5	Al Nasser *et al.* (1992)
Taiwan	Haemodialysis	EIA-2	125	47.2	Sheu *et al.* (1992a)
China	Peritoneal dialysis	EIA	101	29.7	Ng *et al.* (1991)
Japan	Haemodialysis	RIBA	393	17.8	Tamura *et al.* (1992)
Japan	Haemodialysis	ELISA-3	489	41.9	Fujiyama *et al.* (1992)
Spain	Haemodialysis	RIA	42	19.1	Esteban *et al.* (1989)
Australia	Dialysis (unspecified)	ELISA-2	205	5.9	Fairley *et al.* (1990)
	Renal transplantation		261	6.9	
Germany	Haemodyalisis	ELISA	22	9	Abb (1991)

[a]EIA-2, second-generation enzyme immunoassay; ELISA-2, second-generation enzyme-linked immunosorbent assay; RIBA, recombinant immunoblot assay; ELISA C100, ELISA with C100 antigen

examined, duration of intravenous drug use was found to be significantly associated with HCV seropositivity.

1.3.2 *Non-parenteral exposure*

(*a*) *Perinatal*

Inoue *et al.* (1991) reported on a grandmother, mother and baby in Japan, all of whom were seropositive for amplified HCV DNA fragments. The baby developed clinical hepatitis and was seropositive for HCV antibody (C100) in an ELISA; the mother and grandmother had antibodies to the nucleocapsid P22 antigen. A study of the offspring of 17 HCV antibody-seropositive women in Hong Kong (Reesink *et al.*, 1990) revealed only one seropositive for HCV antibody, although six babies of 217 HCV-seronegative women were seropositive; the difference was not significant. A study of 13 children born to nine HCV antibody-seropositive women in Japan (Kuroki *et al.*, 1991) showed that passively transmitted

Table 6. Prevalence of antibodies to HCV in intravenous drug users

Country	Assay[a]	No. of people	% with Ab	Reference
Spain	1st-gen. RIA	83	71.1	Esteban *et al.* (1989)
Australia	ELISA-2	172	86.0[b]	Bell *et al.* (1990)
Australia	ELISA-2	431	61.9	Fairley *et al.* (1990)
Italy	1st-gen. ELISA	80	67.5	Girardi *et al.* (1990)
Germany	1st-gen. ELISA	51	63	Abb (1991)
Sweden	1st-gen. ELISA	172	80	Widell *et al.* (1991)
Netherlands	1st-gen. ELISA	304	73.7	van den Hoek *et al.* (1990)
USA	RIBA	225	85.3	Donahue *et al.* (1991)
Canada	1st-gen. ELISA	76	50.0	Anand *et al.* (1992)

[a]1st-gen. RIA, first-generation radioimmunoassay; ELISA-2, second-generation enzyme-linked immunosorbent assay; RIBA; recombinant immunoblot assay
[b]Among people injecting drugs for more than eight years, there was 100% anti-HCV seropositivity.

antibody persisted up to six months of age; after that age, all of the babies were seronegative but 11 of the 13 children were HCV RNA seropositive. In two of these mother–child pairs, the mother had been transfused after birth and may have acquired HCV by that route. A further study of eight HCV-seropositive women (Thaler *et al.*, 1991) confirmed that passive antibody was lost by nine months of age, but all of the children were HCV RNA seropositive. No relationship was seen with the human immunodeficiency virus (HIV) status of the mother.

A study of the infants of 43 intravenous drug users (Weintrub *et al.*, 1991), using RIBA, showed that 17 children had passive antibody up to the age of four months. Three of 24 initially seronegative infants were persistently seropositive for antibodies to HCV up to 18 months of age.

In a study in Spain of transmission among HIV-seropositive, HCV-seropositive mothers (Perez Alvarez *et al.*, 1992), 21 of 22 children had maternal HCV antibodies, which became undetectable by three months of age. One child had persistent antibodies and went on to develop non-A, non-B hepatitis with HIV infection. The mother of this child had advanced AIDS. In a study of eight pregnant women with HCV RNA detected by PCR (Novati *et al.*, 1992), five of the women were seropositive for HCV (all were also seropositive for HIV). Four of eight children were seropositive for HCV RNA, three of them at birth. One child had persistent viraemia, and the other three were intermittently seropositive for HCV RNA. All three children lost antibody in the same way as the children who were not viraemic.

(b) Familial and household transmission

As HBV is known to be transmitted within the households of carriers, some workers have examined the prevalence of infection in households of people known to be HCV antibody seropositive. In Spain, Menéndez *et al.* (1991) studied 530 household contacts of 225 subjects seropositive for antibodies to HCV: 26 relatives (4.9%) were seropositive—a

significantly greater proportion than among blood donors. There was no difference in prevalence between sexual and non-sexual contacts. The seroprevalence of HCV antibody in the contacts increased with age and was highly correlated with duration of contact with the index patient. A study of household contacts of seropositive haemodialysis patients in Italy (Calabrese *et al.*, 1991) showed 7% of 30 family members to be seropositive. In a second study in Italy (Mondello *et al.*, 1992), household contacts of patients with cirrhosis were examined, comprising eight husbands, eight wives, 44 children and 57 siblings of 21 patients. Two partners (12.5%), five of the children (11.3%) and 27 of the siblings (48.8%) were seropositive for antibodies to HCV (tested by RIBA-2). These prevalences were similar to those of HBV infection in the same families. In Japan, the seroprevalence of HCV antibody (by ELISA) was zero in a survey of 1442 schoolchildren (Tanaka, E. *et al.*, 1992). In Saudi Arabia (Bahakim *et al.*, 1991), however, marked geographical variation in the seroprevalence of antibody was seen in children under 10 years of age: in Riyadh, 0.9%; in Taif, 1.5%; and in Gizan, 5.7%. A report from Canada (Chaudhary *et al.*, 1992) in a home for the mentally handicapped, showed no HCV antibody (by ELISA-2) in a group of 264 children (128 with Down's syndrome), although there was a high rate of HBV infection.

(*c*) *Sexual transmission*

In two studies, HCV RNA was not detected by PCR in the semen of patients seropositive for HCV antibody (by ELISA) and with chronic hepatitis C (Fried *et al.*, 1992; Terada *et al.*, 1992). In contrast, in a study of 34 patients with chronic hepatitis C who were seropositive for HCV RNA, 24% of seminal fluid samples and 48% of saliva samples contained HCV RNA. Subjects who were seronegative for HCV RNA but seropositive for antibodies to HCV had no HCV RNA in body fluids (Liou *et al.*, 1992). An increased frequency of antibodies to HCV (by ELISA) was found in the semen of non-A, non-B hepatitis patients over that in controls (Kotwal *et al.*, 1992b).

Epidemiological evidence of sexual transmission has been sought by studying the prevalence of infection in sexually active people and in sexual partners of infected individuals. In Canada (Anand *et al.*, 1992), 9.3% of homosexual or bisexual men who were also HIV seropositive were found to be HCV seropositive, compared with 6.4% of a similar group who were HIV seronegative. The difference was not significant. A study of homosexual men in Italy (Gasparini *et al.*, 1991) (using first-generation ELISA) found a seroprevalence of HCV infection of 18.9%, but no association was seen with HIV or HBV seropositivity, with the type of intercourse or with sexual promiscuity. The seroprevalence of hepatitis C was 1.6% in a group of 926 homosexual or bisexual men in Baltimore, USA. Only intravenous drug use and a history of hepatitis A were associated with HCV seropositivity; there was no association with HIV-1 seropositivity or sexual behaviour variables (Donahue *et al.*, 1991). Studies of HCV antibody seroprevalence in people attending clinics for sexually transmitted diseases showed an association with such diseases, which is not as strong as that with HIV-1 or HBV (Corona *et al.*, 1991; Ranger *et al.*, 1991; Gutierrez *et al.*, 1992; Schoub *et al.*, 1992).

Studies of seroprevalence in spouses (using antibody assays) have provided little evidence of sexual transmission (Lin *et al.*, 1991; Chan, G.C.B. *et al.*, 1992). In a study of

195 spouses of Japanese patients with HCV-related chronic liver disease (Akahane *et al.*, 1992), those who had had transfusions or a history of hepatitis before marriage were excluded. The remaining 176 were tested for HCV core antibody, HCV RNA and C100 antibody (by ELISA). Of the spouses, 6% were seropositive for C100, 12% were seropositive for core antigen and seronegative for C100, and 18% were seropositive for both; 8% were HCV RNA seropositive. No controls were used in this study, but genotyping of RNA from six spouse pairs showed concordance in all of them. Studies of HCV RNA require careful interpretation.

(*d*) *Population transmission*

A number of researchers have attempted to determine the modes of transmission in populations. Alter, M.J. *et al.* (1989) carried out a case–control study in which the cases were patients with notified acute non-A, non-B hepatitis in two counties of the USA over a 12-month period. People with a known source of infection—a history within the preceding six months of blood transfusion (13%) or intravenous drug use (34%)—were excluded, leaving 74 cases. Matched controls were selected for 52 (70%) of these cases. No increased risk was found for a range of activities, including homosexual activity, health care employment, surgery, dental work or international travel; however, significant odds ratios were found for individuals with $\leq$ 12 years of education, more than two sexual partners and a history of hepatitis in household or sexual contacts. The last two factors were considered to be responsible for 5% and 6% of all non-A, non-B cases, respectively.

Pohjanpelto (1992) enquired about risk factors for transmission from all individuals found to be seropositive for antibodies to HCV (by enzyme immunoassay) in a laboratory in Finland. Information was obtained for 160 of 276 seropositive individuals, of whom 89% reported exposure *via* blood (64% due to intravenous drug use and 24% due to transfusion), 0.6% had a sexual partner seropositive for HCV antibody, and 8.1% had lived or travelled to countries such as Somalia, Egypt and Saudi Arabia. There were no controls in this study. In Texas, USA (van Bohman *et al.*, 1992), 23 pregnant women seropositive for HCV antibody (by ELISA; 18 with confirmation by RIBA) were compared with seronegative women, giving 1005 consecutive births. Seropositivity was significantly associated with intravenous drug use, a history of sexually transmitted disease, a history of HBV infection, sex with an intravenous drug user and more than three sexual partners during life. In a case–control study on blood donors in Sydney, Australia (Kaldor *et al.*, 1992), the cases were people who were repeatedly seropositive for antibodies to HCV (by ELISA and RIBA), and controls were those repeatedly seropositive by ELISA and seronegative by RIBA. Highly significant, independent associations with seropositivity were found for intravenous drug use, having a tattoo and the number of heterosexual contacts. Blood transfusion was not a significant risk factor in this study.

[The Working Group noted that the parenteral route is a major source of infection in some populations. They also noted that sexual and perinatal transmission can occur but that current data do not allow estimation of their relative importance in different populations.]

1.4. Clinical diseases (other than cancer)

HCV is the major cause of parenterally transmitted non-A, non-B hepatitis worldwide. Exposure to this agent often results in a clinically indolent infection, which, however, carries a risk of long-term morbidity (Mondelli & Colombo, 1991; Czaja, 1992).

1.4.1 *Acute infection*

The time-lag between exposure to HCV during transfusion and development of clinical acute hepatitis is 2–26 weeks, with a peak of onset between 6 and 12 weeks (Alter, H.J. *et al.*, 1989). Using second-generation ELISA, which detects serum antibodies against both structural and non-structural proteins of HCV, the mean time between exposure and seroconversion is 2.3 weeks (Mattsson *et al.*, 1992). In patients with transfusion-associated non-A, non-B hepatitis followed prospectively for 10–14 years, the time between exposure to HCV and onset of hepatic virus replication, detected by serum HCV RNA, is as short as one week (Farci *et al.*, 1991). The hepatitis is clinically mild during its acute phase. The range of serum alanine aminotransferase (ALT) levels is 200–600 IU/L, and 75% of cases are anicteric and relatively asymptomatic (Alter, H.J. *et al.*, 1989; Aach *et al.*, 1991). In contrast, community-acquired hepatitis C is more often symptomatic (Alter *et al.*, 1992). A likely explanation for this discrepancy is that studies of community-acquired hepatitis have as their starting point the enrolment of patients with clinically detectable disease. Thus, the occurrence of community-acquired hepatitis C is probably underestimated because of the large number of subclinical cases that escape detection.

One characteristic feature of hepatitis C is a fluctuating serum ALT pattern (Alter H.J. *et al.*, 1989; Mondelli & Colombo, 1991). Patients may have highly fluctuating ALT levels within periods of time as short as one week, and such variations may persist. A smaller number of patients have a single ALT peak and then proceed to apparent full recovery, or a plateau-like, mild elevation of ALT level. Patients with a monophasic pattern of serum ALT recovered from hepatitis more often than patients with either fluctuating or plateau-like serum ALT patterns (Tateda *et al.*, 1979). A feature of hepatitis in some patients is an apparently long-lasting normalization of serum ALT, suggesting full recovery, which is followed later by symptomless enzymatic exacerbations. Antibody against the non-structural C100-3 epitope of HCV (by first-generation ELISA) disappears from almost all patients who recover clinically and biochemically but persists in patients with chronic hepatitis (Alter, H.J. *et al.*, 1989; Alberti, 1991).

While the majority of cases of acute hepatitis C are clinically indolent, severe cases occur. Fulminant hepatic failure is seen rarely in patients who are immunosuppressed or have pre-existing liver disease. On the basis of serum HCV RNA, a marker for replicating virus, HCV may be the cause of hepatic failure in up to 18% of cases of fulminant non-A, non-B hepatitis (Theilmann *et al.*, 1992; Féray *et al.*, 1993). In some other patients with fulminant hepatitis, HCV has been implicated as a cofactor in conjunction with other hepatitis viruses (HAV, HBV or HDV) (Féray *et al.*, 1993) or with drugs.

1.4.2 *Chronic infection*

Twenty percent of all patients with chronic hepatitis C progress to cirrhosis, regardless of the route of infection (Mendenhall *et al.*, 1991; Mondelli & Colombo, 1991).

In many patients, development of chronic liver disease is heralded by persistent elevations in serum ALT activity for more than six months after the onset of acute hepatitis C, and is accompanied by persistence of serum antibodies to HCV and HCV RNA. Histological features of chronic liver disease and cirrhosis have also been detected, however, in seropositive viraemic patients with a persistently normal ALT level (Alberti *et al.*, 1992). In most patients, progression of hepatitis C to cirrhosis is a clinically indolent process, with an average length of approximately 20 years (Kiyosawa *et al.*, 1990a). Even the apparently benign disease, chronic persistent hepatitis C, entails a risk of progression to cirrhosis (Hay *et al.*, 1985). As with tranfusion-associated infection, most patients with HCV acquired by other routes had persistent infection for several years, even in the absence of active liver disease (Alter *et al.*, 1992).

Chronic HCV infection may have important clinical consequences. In a long-term multicentre follow-up study of 568 patients who developed post-transfusion hepatitis between 1967 and 1980, there was a small but significant increase in the number of deaths related to liver disease (Seeff *et al.*, 1992).

The factors that influence the severity of liver damage and the rate of progression to cirrhosis in patients with HCV infection are largely unknown. Clinical and epidemiological factors that may predict the severity of chronic hepatitis C include age at infection, duration of disease, serum ALT levels, co-occurrence of HBV infection and alcoholism. In haemophiliac patients, chronic hepatitis and cirrhosis were more often detected in those with persistently elevated serum levels of ALT (54%) than in individuals with only intermittently abnormal enzyme levels (7%) (Colombo *et al.*, 1988). In a US multicentre study of alcoholics, anti-HCV was more frequently associated with cirrhosis than with less severe hepatic lesions (Mendenhall *et al.*, 1991). Finally, studies of virus genotyping have indicated that the severity of liver disease correlates well with the predominance of the Japanese strain or with the co-occurrence of multiple strains (Takada *et al.*, 1992).

There is controversy about whether the course of HCV infection is different in immunocompromised patients. In a three-year follow-up of 97 patients with non-A, non-B hepatitis (Martin *et al.*, 1989), there was evolution to cirrhosis in 11, including the only three with HIV infection; these three patients developed symptomatic cirrhosis within three years of the onset of hepatitis. The serum titres of HCV RNA were higher in HIV-seropositive than in HIV-seronegative patients (Wright *et al.*, 1992a). Liver-graft infections recurred in almost all patients who were infected with HCV before transplantation, and accelerated hepatic histological deterioration was seen in some (Féray *et al.*, 1992; Wright *et al.*, 1992b).

1.4.3 *Extrahepatic manifestations*

Several extrahepatic manifestations of HCV infection have been described. For instance, 82% of 74 Italian patients with porphyria cutanea tarda had circulating antibodies to HCV (by RIBA) (Fargion *et al.*, 1992). A serum sickness-like syndrome has been described in patients with acute non-A, non-B hepatitis (Perrillo *et al.*, 1981). The possible link betweem HCV and such extrahepatic syndromes as polyarteritis nodosa and idiopathic pulmonary fibrosis is under debate. Antibodies to HCV were detected (by ELISA-2, confirmed by RIBA-2) in three (8%) of 38 patients with polyarteritis nodosa (Deny *et al.*, 1992). Antibodies to HCV were found in 19/66 (29%) Japanese patients with idiopathic

pulmonary fibrosis by ELISA-1, whether or not there was chronic liver disease; RIBA was used to confirm the presence of antibodies in 12/19 patients (Ueda *et al.*, 1992). HCV has been implicated in many cases of type-II cryoglobulinaemia. Agnello *et al.* (1992) detected HCV RNA in 16/19 such patients and antibody to HCV in eight (by RIBA). Quantitative studies in four patients showed that almost all of the HCV RNA sequences were concentrated in the cryoprecipitate. Eight patients with membranoproliferative glomerulonephritis had circulating antibodies to HCV (by RIBA), and cryoglobulin-like structures, immunoglobulin G and M and C3 antigen were demonstrated within the glomeruli (Johnson *et al.*, 1993). In another study, 57% of 28 patients with chronic hepatitis C had histological evidence of Sjøgren's syndrome, compared with only 5% of controls with miscellaneous diseases (Haddad *et al.*, 1992).

1.5 Therapy

No vaccine is currently available for HCV infection.

1.5.1 *Acute and fulminant HCV infection*

Most cases of acute HCV infection are asymptomatic and do not require medical attention. In cases of malaise and fatigue, bed rest is advised. In symptomatic acute HCV infection, therapy is aimed at relief of the signs and symptoms associated with the acute phase of the disease. It includes parenteral nutrition in cases of dehydration and inanition due to nausea and vomiting, and replacement of coagulation factors in cases of bleeding due to impaired synthetic liver function. While fulminant hepatitis C is a rare clinical entity (Wright *et al.*, 1991; Féray *et al.*, 1993; Liang *et al.*, 1993), liver transplantation is a therapeutic option in advanced liver failure and hepatic coma (Maddrey & Van Thiel, 1988). Few trials of antiviral agents in acute HCV infection have been carried out with the intention of preventing the progression of acute hepatitis C to chronic liver disease. While in a study from Japan, natural β-interferon appeared to be effective (Omata *et al.*, 1991), a study from Spain involving recombinant α-interferon showed no benefit for the long-term outcome of the disease (Viladomiu *et al.*, 1992).

1.5.2 *Chronic HCV infection*

Because of the potentially severe natural course of HCV infection, several therapeutic strategies have been explored. While ribavirin therapy did not significantly affect HCV replication (Di Bisceglie *et al.*, 1992), administration of α-interferon three times per week (Davis *et al.*, 1989; Di Bisceglie *et al.*, 1989; Ruiz-Moreno *et al.*, 1992; Shindo *et al.*, 1992a) or continuously (Carreño *et al.*, 1992) was effective in about 50% of patients, resulting in normalization of liver function, disappearance of HCV RNA from serum and improved liver histology. Unfortunately, about 50% of patients suffer a relapse after cessation of therapy, with increased serum transaminase levels and reappearance of HCV RNA (Davis *et al.*, 1989; Di Bisceglie *et al.*, 1989; Carreño *et al.*, 1992; Garson *et al.*, 1992a; Ruiz-Moreno *et al.*, 1992; Shindo *et al.*, 1992a). A long-term response to α-interferon therapy therefore occurs in only about 25% of patients with chronic HCV infection. Studies are under way to explore the benefit of long-term therapy of chronic HCV infection with α-interferon.

The parameters that predict a response to α-interferon therapy are not well defined (Black & Peters, 1992). In contrast to HBV infection, co-infection with HCV and HIV does not seem to reduce the efficacy of α-interferon therapy (Boyer *et al.*, 1992), but patients immunosuppressed after organ transplantation respond poorly to α-interferon therapy (Davis, 1989; Wright *et al.*, 1992). Recent evidence suggests that response to α-interferon therapy may be related to the viral genotype and viral load in a given individual with HCV infection (Kanai *et al.*, 1992; Yoshioka *et al.*, 1992). The issue is further complicated by the fact that different genotypes have been identified within individuals (Martell *et al.*, 1992; Murakawa *et al.*, 1992; Tanaka T., *et al.*, 1992; Weiner *et al.*, 1992).

2. Studies of Cancer in Humans

2.1 Case series

Table 7 summarizes the seroprevalence of antibodies to HCV in 30 series of patients with hepatocellular carcinoma (HCC). In most, the prevalence was determined by first-generation assays. In general, the prevalence is high among Japanese cases, relatively high in European populations and relatively low in Chinese and Africans. In those series in which data were included for cases grouped by HBV surface antigen (HBsAg) status, the prevalence of antibodies to HCV was generally substantially higher among the HBsAg-seronegative cases.

2.2 Cohort studies

Kiyosawa *et al.* (1990b) studied 58 patients (41 men and 17 women) with chronic hepatitis C (by first-generation ELISA) admitted to Shinshu University Hospital, Japan, between January 1970 and April 1990. All had a history of blood transfusion, and 20 had had clinical acute hepatitis in the past. Twenty-six patients were diagnosed at the time of entry into the study with chronic active hepatitis and 28 with chronic persistent hepatitis. Serum samples were collected serially for a mean of 13.2 years. Among 54 patients who remained seropositive for HCV antibodies throughout the follow-up period, 10 (18.5%) developed HCC. Among four patients who converted from seropositivity to seronegativity, there was no case of HCC. In a further study including some of these patients, Yousuf *et al.* (1992) reported on 16 of 62 HCV-seropositive patients who developed HCC after a mean interval of 9.5 years. [Insufficient detail was provided on the selection of the cases, and no information was given on length of follow-up between the two comparison groups, precluding calculation of expected numbers.]

Seeff *et al.* (1992) studied 545 patients with post-transfusion non-A, non-B hepatitis (diagnosed by exclusion) and 930 matched controls who had received transfusions but did not develop non-A, non-B hepatitis. The subjects were drawn from among participants in five prospective studies of post-transfusion non-A, non-B hepatitis in the USA conducted between 1967 and 1980. Of the 568 cases, 76% were male. The 984 controls were similar to cases with respect to age, sex, race, treatment centre, receipt of immune globulin, history of alcoholism, number of units of blood transfused and date transfused. Cause of death was

Table 7. Prevalence of antibodies to HCV in case series of patients with hepatocellular carcinoma (HCC)

Reference	Location	Period	Assay	Prevalence of antibodies to HCV						Comments
				All HCC patients		HBsAg-positive patients		HBsAg-negative patients		
				Total	%	Total	%	Total	%	
Africa										
Levrero *et al.* (1991)	Senegal	1980–88	ELISA	93	NR	NR	27	NR	68	
Robson *et al.* (1991)	South Africa	NR	ELISA and confirmation by Abbott neutralization EIA; C100-3	30	7	11	37	NR		
Bukh *et al.* (1993)	South Africa	1987–90	HCV RNA and 2nd-gen. ELISA	128	20	71	10	57	33	
Americas										
El-Ashmawy *et al.* (1992)	USA	1985–88	Confirmation by RIBA	38	26	11	45	27	19	Liver transplant patients
McHutchison *et al.* (1992)	USA	NR	1st- and 2nd-gen. EIA and RIBA	46	52	NR		NR		
Asia										
Kiyosawa *et al.* (1990a)	Japan	1958–89		83	73	29	35	54	94	21 patients with a history of transfusion all seropositive before HCC developed
Ohkoshi *et al.* (1990)	Japan	NR	ELISA	100	58	42	19	58	86	
Nishioka *et al.* (1991)	Japan	NR	ELISA	180	51	75	15	105	76	
Watanabe *et al.* (1991)	Japan	NR	ELISA (C100) P22	125 125	55 69	23 23	4 4	102 102	67 83	

Table 7 (contd)

Reference	Location	Period	Assay	Prevalence of antibodies to HCV						Comments
				All HCC patients		HBsAg-positive patients		HBsAg-negative patients		
				Total	%	Total	%	Total	%	
Asia (contd)										
Hagiwara *et al.* (1992)	Japan	NR	C100-3	NR		NR		39	74	All cases HBsAg-seronegative; 5/10 seronegatives were HCV RNA seropositive by PCR
Lee *et al.* (1992)	China	NR	ELISA	326	13	243	4	83	37	
Leung *et al.* (1992)	Hong Kong	1986–90	ELISA	424	7	341	4	83	19	Male to female ratio,7:1
Shimizu, S. *et al.* (1992)	Japan	1985–89	2nd-gen. ELISA (83%) and RIBA (58%)	NR		NR		24	58	All cases were in alcoholics
Yuki *et al.* (1992)	Japan	NR	C100-3	148	70	38	32	110	83	
Chien *et al.* (1992)	Japan	NR	ELISA (C100-3) C25 or C100-3	NR NR		NR NR		268 268	63 83	
Sheu *et al.* (1992b)	China	1988–90	1st- and 2nd-gen. assays	NR		NR		31	68	
Kiyosawa & Furuta (1992)	Japan	1971–80 1981–90	NR	112 267	34 59	65 86	8 5	47 181	81 88	
Takeda *et al.* (1992)	Japan	1980–89	ELISA	100	51	27	11	73	66	
Sun *et al.* (1993)	China	NR	2nd-gen. ELISA	112	5	NR		NR		

Table 7 (contd)

Reference	Location	Period	Assay	Prevalence of antibodies to HCV						Comments
				All HCC patients		HBsAg-positive patients		HBsAg-negative patients		
				Total	%	Total	%	Total	%	
Europe										
Colombo *et al.* (1989)	Italy	1975–88	ELISA (C100-3)	132	65	41	54	91	70	
Simonetti *et al.* (1989)	Italy	1982–88	ELISA	200	76	31	58	169	79	
Amitrano *et al.* (1990)	Italy	1989	ELISA	29	62	NR		NR		
Sbolli *et al.* (1990)	Italy	1981–89	ELISA	78	61	8	13	70	64	
Vargas *et al.* (1990)	Spain	NR	NR	81	54	NR		NR		
Benvegnù *et al.* (1991)	Italy	NR	2nd-gen. RIBA	40	65	NR		NR		14 seropositives negative in 1st-gen. assay
Levrero *et al.* (1991)	Italy	1980–88	ELISA	74	NR	NR	30	NR	76	
Nalpas *et al.* (1991)	France	1982–89	ELISA	55	58	12	75	35	57	
Farinati *et al.* (1992)	Italy	NR	2nd-gen. ELISA and RIBA	97	64	NR	43	NR		
Garson *et al.* (1992b)	Switzerland	NR	2nd-gen. EIA and RIBA	40	35	7	14	33	39	
Baur *et al.* (1992)	Austria	NR	ELISA	54	22	22	18	32	25	Any HBV marker

NR, not reported; HBsAg, HBV surface antigen; ELISA, enzyme-linked immunosorbent assay; EIA, enzyme immunoassay; RIBA, recombinant immunoblot assay; PCR, polymerase chain reaction

determined from death certificates for 545 (96.0%) of the cases and 930 (94.5%) controls as at February 1992, giving an average of 18 years of follow-up. No testing for antibodies to HCV was done. One case of HCC was found in the hepatitis group and two in the controls [estimated RR, 1.0]. [There were too few cases of HCC for the study to be informative.]

Verbaan *et al.* (1992) followed 566 patients (331 men and 235 women), with a mean age at entry into the study of 52.1 years, in a hospital in Malmö, Sweden, from whom liver biopsy samples were taken for assessment of chronic liver disease between 1978 and 1989. Causes of death were obtained from death certificates or autopsy records. Sera stored at the time of biopsy were tested for antibodies to HCV by first- and second-generation ELISA, and sera positive in these two tests were retested by second-generation RIBA. Of the 566 patients, 78 (13.8%) were seropositive by RIBA. Eleven cases of HCC developed over the follow-up period; two were diagnosed at the time of inclusion in the study. The proportion of deaths due to HCC in the seropositive group (5/23, 22%) was significantly different ($p = 0.01$) from that in the seronegative group (6/130, 5%).

In a cohort study from Taiwan, China, serum samples from 9691 male adults were collected and frozen during 1984–86 (Yu & Chen, 1993). A total of 35 cases of HCC were identified between 1984 and 1990 and matched individually by age, time of sample collection and residence to two HBsAg-seropositive and two HBsAg-seronegative controls from the original cohort. Samples were analysed for HBsAg status by a radioimmunoassay, for antibodies to HCV by an enzyme immunoassay and for serum testosterone level. Seven of the 35 cases and four of the 140 controls were seropositive for antibodies to HCV [crude relative risk (RR), 9] (multivariate-adjusted RR, 12; 95% confidence interval (CI), 2.4–58).

2.3 Case–control studies

For those studies in which estimated odds ratios (OR) are not provided, the Working Group calculated them using the crude data. The estimates are therefore not adjusted for other factors.

2.3.1 *First-generation assays*

Table 8 (p. 191) gives a summary of the case–control studies in which first-generation antibody tests were used (see section 1.2) and includes available information on the study period and source of control subjects.

(a) Africa

Coursaget *et al.* (1990) reported on 80 cases of HCC and 136 adult controls in Senegal. Sera were collected between 1982 and 1986. The seroprevalence of antibodies to HCV was 37.5% among the cases and 3% among the controls [OR = 20]. [No data were given on the age and sex distribution of subjects nor how they were selected.]

Kew *et al.* (1990) studied 380 southern African blacks (322 men, 58 women) with histologically confirmed HCC and compared them with 152 controls matched for race, sex, age and rural or urban status. The seroprevalence of antibodies to HCV among the cases (29%) was higher than that among the controls (1/152) [OR, 62]. Of the 196 HBsAg-seronegative cases, 32% were seropositive for HCV antibodies, compared with 26% of the 184 HBsAg-seropositive cases. [The periods of collection of data and sera were not given.]

(*b*) *Americas*

In a study conducted in the USA, Hasan *et al.* (1990) studied retrospectively a total of 87 HCC patients who had been diagnosed between January 1978 and March 1989 at the University of Miami Hospital and Clinic. Diagnosis was made either histologically, cytologically or by level of serum α-fetoprotein with at least one positive imaging study. Cases with alcoholic liver disease, haemochromatosis or α_1-antitrypsin deficiency were excluded. Controls were 200 consecutive blood donors. Forty percent of the cases and 0.5% of the controls were seropositive for antibodies to HCV (tested by the method of Kuo *et al.*, 1989, see p. 168) [OR, 134]. The seropositivity among cases varied by ethnicity, being found among the HBsAg-seronegative patients in 35% of 37 whites, 80% of 20 hispanics and the one black; the only Asian was seronegative. The seroprevalence of antibodies to HCV among the 41 cases with no evidence of past HBV infection was 49%, that among the 18 seropositive only for anti-HBc was 61%, and that among the 28 cases who were HBsAg seropositive was 14%. All cases who were HCV antibody seropositive and HBsAg seronegative had evidence of cirrhosis. [The sex and age distribution of the HBsAg-seropositive cases and of the controls were not given. It was not evident when the case blood samples were collected in relation to diagnosis or whether case and control samples were collected at different times.]

Yu *et al.* (1990) evaluated data on 51 cases (in 35 males and 16 females; mean age, 59.5 years) and 128 controls (81 males; mean age, 58.7 years) obtained in 1984–89 from Los Angeles County, California (USA) (for details, see p. 80 of the monograph on HBV). Of the cases, 29% were seropositive for antibodies to HCV, as were 4% of the controls. An OR of 11 was found for the association between HCV antibody seropositivity and HCC after adjustment for age and sex. The OR for antibodies to HCV in subjects with no HBV markers was 4.8; that for any HBV marker among the HCV seronegative subjects was 4.4. Ten cases and no control had evidence of any serological marker of HBV infection and were HCV seropositive ($p < 0.0005$). The authors noted that the serum specimens of the controls were drawn on average six years before those of the cases.

Di Bisceglie *et al.* (1991a) studied 99 cases of HCC seen at Johns Hopkins Oncology Center, Baltimore, USA, between January 1987 and May 1988 (for details, see p. 81 of the monograph on HBV). Controls consisted of 98 consecutive adult patients with other cancers seen between November 1987 and January 1988. The seroprevalence of antibodies to HCV was 13% among the cases and 2% among the controls; the OR for an association with HCC was 7.3.

(*c*) *Asia*

Saito *et al.* (1990) studied the seroprevalence of antibodies to HCV among 253 (207 men and 46 women; mean age, 61 years) patients with HCC diagnosed clinically and pathologically in several hospitals in Japan and from whom blood specimens were obtained at the time of diagnosis. For comparison, they evaluated 148 patients with other cancers (95 men, 53 women; mean age, 61.1 years). The seroprevalence of antibodies to HCV was 55% among cases and 10% among controls [OR, 11]. The prevalence among the cases varied by HBV status. The authors noted an association between history of transfusion and seropositivity for HCV antibodies in the controls [$p = 0.003$] but not in the cases, and that the control rate was

higher than that seen in Japanese blood donors (about 1%). [No data were given on the period of study.]

In a case–control study in Qidong County, Jiangsu, China, 50 cases of HCC diagnosed during 1988 were compared with 50 population controls individually matched to cases by age, sex and place of residence (Xu *et al.*, 1990). Four cases and no control were seropositive for antibodies to HCV ($p = 0.059$); one of these cases was seronegative for HBsAg, while the remaining three were seropositive.

Jeng and Tsai (1991) studied 48 HCC patients (35 men, 13 women; mean age, 62.0 years) in Taiwan, China, who were HBsAg seronegative and who had been recruited after admission to Kaohsiung Medical College Hospital between January 1988 and June 1990. Diagnosis was made histologically or cytologically. Controls were 54 HBsAg-seronegative individuals (46 men, eight women; mean age, 52.3 years) who were seen for normal physical check-ups during the same period. Seroprevalence for antibodies to HCV differed significantly between the two groups (60% *versus* 0 [$p = 0.0001$]). The authors also reported a significant difference between HCV antibody seroprevalence in these cases and in 81 HBsAg-seropositive HCC cases seen during the same period (23.5%; $p = 0.0001$).

Srivatanakul *et al.* (1991) conducted a matched case–control study on HCC in cases seen in Thailand in 1987–88 (Parkin *et al.*, 1991; described in detail on p. 85 of the monograph of HBV). There was no association between the presence of antibodies to HCV and HCC (OR, 1.3); the prevalence among cases was 6% and that among the controls was 5%.

Yu *et al.* (1991) conducted a matched case–control study in Taiwan, China, of HCC cases from two major teaching general hospitals newly diagnosed between August 1986 and July 1987 on the basis of either pathological examination or serum α-fetoprotein levels, confirmed by imaging. There were 127 cases (121 men, 6 women; mean age, 50.4 years). Controls were selected from household registration lists, were matched for age, sex, ethnicity and residence and were recruited during the same period. The seroprevalence of antibodies to HCV was 11% among the cases and 2% among the controls. In a matched analysis, the univariate estimate of the OR associated with seropositivity for HCV antibody was 7.0, and the ratio remained elevated after control for HBsAg status, HBeAg status, smoking habits, habitual alcohol use and peanut consumption. The seroprevalence of antibodies to HCV was higher in the 17 HBsAg-seronegative cases (29%) than in the 110 HBsAg-seropositive cases (8%). Among the HBsAg-seronegative subjects, the OR for HCV seropositivity and HCC was 16. The OR for co-infection as compared with seropositivity for neither marker could not be estimated, with nine cases and no control observed.

HCV antibody status was measured in 1989–90 using an enzyme immunoassay in 42 cases of HCC and in 4818 blood donors enrolled from the Military Hospital in Riyadh, Saudi Arabia (Al Karawi *et al.*, 1992). The seroprevalence of antibodies to HCV was 31% in cases and 1.5% among blood donors [crude OR, 30; 95% CI, 15–60]. The seroprevalence of antibodies to HCV was higher among HBsAg-seronegative HCC cases (42%) than among seropositive HCC cases (13%). [No information was available on the age or sex of the study subjects.]

In a study that overlapped partially with that of Yu *et al.* (1991), Chuang *et al.* (1992) studied 128 cases of HCC (in 112 men, 16 women; mean age, 54.3 years) and 384 age- and

sex-matched community controls in Taiwan, China (described on p. 85 of the monograph on HBV). Twenty percent of the cases and 3% of controls were seropositive for HCV antibodies. Among the cases, the seroprevalence was 45% for the 29 who were HBsAg seronegative and 12% for the 99 who were HBsAg seropositive. The OR for HCC was 27 among the subjects seropositive for HCV antibody and HBsAg seronegative. The OR for HCC for the joint presence of HBsAg and HCV antibody seropositivity as compared with the presence of neither was 40. [The period of data collection was not given.]

(*d*) *Europe*

The first study on HCV and HCC, reported by Bruix *et al.* (1989), was conducted in Barcelona, Spain. The cases were 96 (67 men, 29 women; mean age, 63.4 years) consecutive patients with HCC confirmed by ultrasonography and biopsy or with elevated serum α-fetoprotein level. The control group comprised 177 hospitalized surgical controls without liver disease (119 men, 58 women; mean age, 54.3 years). The seroprevalence of antibodies to HCV was 75% among cases and 7% among controls. Among cases, the seroprevalence varied somewhat by risk category: all of four cases with porphyria and cirrhosis, 81% of 43 with cirrhosis of unknown etiology, 77% of 30 with alcoholic cirrhosis, 56% of nine HBsAg-seropositive cases, none of three with a previously normal liver and 71% of seven with previous liver status unknown. The seroprevalence of antibodies to HCV in the case group was significantly higher than that in the control group without liver disease [OR, 38]. [Data on time period of serum collection were not provided.]

Caporaso *et al.* (1991) reported on 332 consecutive patients with cirrhosis seen in a medical centre in Naples, Italy, between January 1988 and May 1990. HCC was diagnosed in 88 of these by ultrasonographic examination and serum α-fetoprotein levels and/or by cytology. The patients with HCC were more likely than the 244 control patients with only cirrhosis to be male (88 *versus* 61%) and to be older (mean age, 64.5 years *versus* 57.0). The seroprevalence of antibodies to HCV among the cases was 72% and that among the controls was 55% [RR, 2]. After control for age and sex, seropositivity for antibodies to HCV was a significant predictor of HCC ($p = 0.009$).

Poynard *et al.* (1991) studied 2015 patients admitted in 1982–89 to the hepatogastroenterology service of the Antoine Béclère Hospital in Clamart, France, for alcoholism and alcoholic liver disease, using a uniform protocol. All patients were classified as alcoholic on the basis of a history of consumption of at least 50 g alcohol per day in the year before admission. Serum samples were available for testing for antibodies to HCV from 469 patients with documented liver cirrhosis (51 HCC cases and 418 controls). Diagnosis was made histologically or on the basis of α-fetoprotein levels, confirmed by ultrasonography and other imaging. The presence of cirrhosis was determined in all subjects either by biopsy or by use of an established algorithm. The seroprevalence of antibodies to HCV was 41% among the cases and 26% among the controls, giving an OR for an association with HCC of [2.0]; this estimate did not vary markedly with the presence of HBsAg. On multivariate analysis, age, male sex, HBsAg seropositivity and HCV antibody seropositivity were all significant risk factors for HCC; HCV antibody seropositivity was the weakest ($p = 0.04$). [Sex ratio and age range within the group were not available.]

Tzonou *et al.* (1991) re-evaluated subjects from an earlier case–control study (Trichopoulos *et al.*, 1987; described on p. 88 of the monograph on HBV). As reported previously (Kaklamani *et al.*, 1991), only strong seropositivity for antibodies to HCV (twice the recommended limit) was specifically related to HCC; weakly positive results were related to cases of metastatic liver cancer, suggesting that weakly positive results were false-positives. In the present report, subjects were categorized as strongly positive, weakly positive or negative. For 185 HCC cases and 432 hospital controls, the prevalence of strong reactivity to HCV antibodies was 39% among the cases and 7% among the controls: the OR was 6.2 after adjustment for age, sex, smoking and HBsAg status. The joint presence of seropositivity for HCV antibodies and HBsAg gave an OR of 20; that for HCV-seropositive and HBsAg-seronegative subjects was 4.8 ($p < 0.05$). The prevalence of antibodies to HCV was lower among the 99 HBsAg-seronegative cases (28%) than among the 85 HBsAg-seropositive cases (51%).

Simonetti *et al.* (1992) evaluated 212 consecutive HCC patients (161 men, 51 women; mean age, 62.4 years) admitted to a hospital in Palermo, Italy, between June 1982 and December 1988. Diagnosis was based on biopsy or serum α-fetoprotein level, confirmed by ultrasonography or tomography. Controls were matched for age and sex and were drawn from among patients hospitalized for chronic non-hepatic disease during the same period (mean age, 62.2). Of the cases, 71% were seropositive for antibodies to HCV compared with 5% of the controls; the OR for an association with HCC was 69 after control for markers of HBV. Of the cases, 197 had confirmed cirrhosis (based on clinical criteria in 60 cases and by biopsy in 137 cases), and a second comparison group was assembled of 197 patients with cirrhosis matched for age and sex (mean age, 61.5), who were hospitalized during the same period. Of the cirrhotic cases of HCC, 74% were seropositive for HCV antibody, as were 62% of their controls; the OR for an association with HCC was 2.0 after control for markers of HBV and alcohol abuse.

2.3.2 *Second-generation assays*

Table 9 summarizes the case–control studies in which antibody to HCV was screened using second-generation assays.

(*a*) *Africa*

A case–control study was carried out in Maputo, Mozambique, on 178 HCC patients admitted to the department of gastroenterology of the central hospital of that city and 194 blood donors from the same hospital (Dazza *et al.*, 1993). HCV antibody status was investigated using a second-generation enzyme immunoassay with confirmation by a line immunoassay. Eleven cases and four controls were seropositive for HCV antibody, yielding an age-adjusted OR of 1.1, which was substantially different from the crude OR of 3.1. The OR for seropositivity to HCV antibody was 1.4 (95% CI, 0.4–5.3) among HBsAg-seronegative individuals. The mean age of cases was 40.8 years and that of the controls, 31.3 years; the mean age of HCV antibody-seropositive cases was 53.9 and that of seronegative cases was 35.1. [The controls were substantially younger than the cases, but the age ranges were not given, and the statistical methods used to control for age were not described.]

Table 8. Summary of results of case–control studies of hepatocellular carcinoma and the prevalence of antibody to HCV as measured by first-generation assays

Reference and location	Subjects	Seroprevalence of antibodies to HCV				OR[a]	95% CI	Study period and comments
		Cases		Controls				
		No.	%	No.	%			
Africa								
Coursaget *et al.* (1990); Senegal	NR	80	37.5	136	3	[20]	[6.9–57]	1982–86; stringent cut-off used for assay
Kew *et al.* (1990); South Africa	Men and women	380	29	152	0.7	[62]	[11–353]	Unmatched hospital controls
Americas								
Hasan *et al.* (1990); USA	NR	87	40	200	0.5	[134]	[23–787]	Cases, 1978–89; unspecified for controls; blood donor controls
Yu, *et al.* (1990); USA	Men and women	51	29	128	4	11	3.5–31	Cases, 1984–89; community controls, 1978–82; all estimates adjusted for age and sex
	Subjects with no evidence of HBV infection					4.8	1.3–18	
	Subjects with any HBV marker					∞	15–∞	
Di Bisceglie *et al.* (1991a); USA	Men and women	99	13	98	2	7.3	1.8–48	Cases, 1/1987–5/1988; controls, 11/1987–1/1988. Controls, other cancer patients
Asia								
Saito *et al.* (1990); Japan	Men and women	253	55	148	10	[11]	[5.9–19]	Controls, other cancer patients. Among controls, significant association of HCV seroprevalence and history of blood transfusion ($p = 0.003$)
Xu *et al.* (1990); China	Men and women	50	4	50	0	∞		Population controls ($p = 0.06$)

Table 8 (contd)

Reference and location	Subjects	Seroprevalence of antibodies to HCV				OR[a]	95% CI	Study period and comments
		Cases		Controls				
		No.	%	No.	%			
Asia (contd)								
Jeng & Tsai (1991); Taiwan, China	Men and women	48	60	54	0	∞		[$p < 0.0001$]; 1988–90; non-hospital controls. 11 subjects were HBsAg seronegative
Srivatanakul *et al.* (1991); Thailand	Men and women	63	6	63	5	1.3	0.2–8.7	1987–88; hospital controls; matched analysis; subjects matched on sex, age, area of residence, hospital
Yu *et al.* (1991); Taiwan, China	Men and women	127	11	127	2	7.0	1.6–31	1986–87; community controls; matched analysis; subjects matched for age, sex, ethnicity and residence
						24	2.2–254	Conditional multivariate analysis with adjustment for matching factors plus HBsAg status, HBeAg status, smoking, habitual alcohol use and peanut consumption
	HCV seropositive/HBsAg seropositive *versus* neither					∞	14–∞	
Al Karawi *et al.* (1992); Saudi Arabia	NR	42	31	4818	1.5	[30]	[15–60]	1989–90; blood donor controls; no data on age or sex
Chuang *et al.* (1992); Taiwan, China	Men and women	128	20	384	3	[6.9]	3.5–14	Community controls
	HBsAg seronegative					27	9.8–75	
	HBsAg seropositive, HCV seropositive *versus* neither					40	13–128	
Europe								
Bruix *et al.* (1989); Spain	Men and women	96	75	177	7	[38]	[18–133]	Hospital controls
Caporaso *et al.* (1991); Italy	Men and women	88	72	244	55	[2.0]	[1.2–3.4]	1988–90; all subjects had cirrhosis

Table 8 (contd)

Reference and location	Subjects	Seroprevalence of antibodies to HCV				OR[a]	95% CI	Study period and comments
		Cases		Controls				
		No.	%	No.	%			
Europe (contd)								
Poynard *et al.* (1991); France	Men and women	51	41	418	26	[2.0]	[1.1–3.7]	1982–89; all subjects were alcoholics and had cirrhosis.
Tzonou *et al.* (1991); Greece	Men and women	185	39	432	7	6.2	3.6–11	1976–84; hospital controls; all estimates adjusted for age, sex, residence; seropositivity for HCV based on 'strongly positive' results
	HCV seropositive/HBsAg seropositive *versus* neither					20	2.5–158	
	HBeAg seropositive or anti-HBe seropositive *versus* neither					∞	5.8–∞	
	HCC with cirrhosis					11	5.3–25	
Simonetti *et al.* (1992); Italy	Men and women Hospital controls	212	71	212	5	69.1	15–308	1982–88; conditional multivariate analysis with control for sex and age plus HBsAg and anti-HBc status
	Controls with cirrhosis	197	74	197	62	2.0	1.3–3.2	1982–88; conditional multivariate analysis with control for age and sex plus HBsAg and anti-HBc status and alcohol abuse

NR, not reported

[a]Cornfield limits; all estimates are unadjusted unless otherwise specified.

A series of 49 cases of HCC and 134 adult controls from the general population were re-investigated in Senegal with regard to HCV antibody status using both a first-generation ELISA test for C100 and an anti-core/anti-C33c recombinant assay (Coursaget *et al.*, 1992). Seropositivity to HCV antibody was found in six (12%) cases and two (1.5%) controls [crude OR, 9.2; 95% CI, 1.8–47]; seropositivity was confirmed by neutralization assay in two cases and one control [crude OR, 5.7]. [No details were provided on the methods or time of recruitment of cases and controls or on potential confounding variables.]

(*b*) *Asia*

Tanaka *et al.* (1991) conducted a case–control study in Fukuoka, Japan, on 91 cases of HCC (in 73 men and 18 women; median ages, 59.0 and 56.5 years, respectively) in 1985–89. A total of 410 controls (291 men, 119 women; median ages, 57.0 and 56.0, respectively) were identified during examinations at public health centres in 1986–89. Most of the cases had evidence of pre-existing liver disease (76 cirrhosis, nine chronic hepatitis). The prevalence of antibodies to HCV was measured with a first-generation enzyme immunoassay, and positive sera were re-tested using a second-generation assay (RIBA). The seropositivity rates in the initial assay were not related to duration of storage of the sera. Seropositivity to HCV antibody was 51% among cases and 3% among controls. The OR for an association with HCC was 52 after adjustment for demographic factors. The seroprevalence of antibodies to HCV was low among patients who were HBsAg seropositive (5%).

In a case–control study carried out in Hanoi, Viet Nam, described in detail in the monograph on HBV (p. 85), HCV antibody status was investigated using a confirmatory line immunoassay (Cordier *et al.*, 1993). Seropositivity to HCV antibody was seen in three of 152 male cases and two of 241 male hospital controls (OR, 2.0). All were HBsAg seronegative (OR, 38; 95% CI, 2.8–1443).

(*c*) *Europe*

Stroffolini *et al.* (1992) studied 65 cases of HCC diagnosed in four teaching hospitals in Palermo, Italy, between January and December of 1990 (described in detail on p. 89 of the monograph on HBV). All cases had cirrhosis; controls were hospitalized patients with non-hepatic chronic diseases. The seroprevalence of antibodies to HCV was determined using a second-generation enzyme immunoassay; positive samples were confirmed by RIBA. Seropositivity to HCV antibody was seen in 66% of cases and 13% of controls (OR, 27 after control for age, gender and HBV markers). Five cases and no control were seropositive for both HCV antibody and HBsAg (OR, 77); the OR for HCV antibody seropositivity alone was 21 and that for HBsAg alone, 13.

Zavitsanos *et al.* (1992) re-tested serum samples obtained during the case–control study described above in Athens, Greece (Tzonou *et al.*, 1991; see also p. 88 of the monograph on HBV). Samples from 181 HCC cases, 35 patients with metastatic liver cancer and 416 hospital controls with no malignant neoplasm or liver disease were examined. The sera had been collected between April 1976 and October 1984 at nine major hospitals in Athens. In the present study, sera were tested by second-generation enzyme immunoassay with confirmation empirically determined using a variety of supplemental enzyme immunoassays with other recombinant peptides and an inhibition assay using C100; a random sample of 32 HCC

patients and 13 hospital controls was also tested by RIBA-2. The authors again assumed that the cases of metastatic liver cancer were true negatives. The final algorithm for positivity was repeated reactivity to the second-generation enzyme immunoassay at an absorbance to cut-off ratio of ≥ 3.0, with confirmation based on supplemental assays with seropositivity for antibody against another viral protein or by inhibition. On the basis of these tests, the OR for an association with HCC was 10.

2.4 Modifying effects of seropositivity for hepatitis B surface antigen

At least six case–control studies presented results according to HCV antibody status (assessed by first-generation tests) separately for HBsAg-seropositive and HBsAg-seronegative individuals, allowing analyses of the separate effects of each virus and their combined effects. A further five studies presented similar results based on second-generation tests (Table 10). With both the first- and second-generation tests, the ORs for HCC in subjects infected with HBV or HCV alone are increased. The OR associated with HCV alone is in general higher in studies based on second-generation tests [combined results: OR, 26; 95% CI, 16–43] than in other studies [combined results: OR, 14; 95% CI, 10–20]. The combined effect of the two viruses cannot be described accurately, given the small numbers of subjects.

The results summarized in Table 10 should be considered with caution, since the estimated ORs were not adjusted for confounding factors such as sex and age. Comparisons between the columns of the table may be valid if it can be assumed that the uncontrolled confounding effect of sex, age and other possible factors varies across the exposure categories.

3. Studies of Cancer in Experimental Animals

Primate

A seven-year-old male chimpanzee (*Pan troglodytes*), seronegative for HBsAg, anti-HBs and anti-HBc and seropositive for antibodies to hepatitis A virus, was inoculated with 40 ml of serum from a human patient with chronic non-A, non-B hepatitis, who was seronegative for markers of HBV and hepatitis A virus, and 10 months thereafter with 10 ml of the chimpanzee's own acute-phase serum (taken at day 34 after inoculation). Over the next six years, the chimpanzee received inoculations of several different plasma-derived products, including concentrates of coagulation factors II, VII, VIII, IX and XIII, and anti-thrombin III. Serum levels of aspartate and alanine transferase, γ glutamyl transferase and HBV markers were monitored and liver biopsies were performed. Serum transferase levels increased after the first inoculation of the human serum and the animal's acute-phase serum. Over the next six years, the levels fluctuated above normal, and serial liver biopsies showed changes ranging from histologically normal to moderate hepatitis (which included focal hepatocellular necrosis and chronic portal inflammation). The chimpanzee remained seronegative for hepatitis B markers throughout the study. Seven years after the first inoculation, liver masses were palpable, and necropsy revealed two large (16×8 cm and 5×7 cm)

Table 9. Summary of results of case–control studies of hepatocellular carcinoma and the presence of antibody to HCV as measured by second-generation assays

Reference and location	Subjects	Seroprevalence of antibodies to HCV				OR[a]	95% CI	Study period and comments
		Cases		Controls				
		No.	%	No.	%			
Africa								
Dazza *et al.* (1993); Mozambique	NR	178	6	194	2	1.1	0.4–3.1	Blood donor controls; adjusted for age; mean age of cases, 40.8 years; controls, 31.3 years
Coursaget *et al.* (1992); Senegal	NR	49	4	134	1	[5.7]	[0.5–69]	General population controls
Asia								
Tanaka *et al.* (1991); Japan	Men and women	91	51	410	3	52	24–114	Cases, 1985–89; controls, 1986–89; general population controls; adjusted for age, sex, occupational class and education; initial screening by first-generation with confirmation by RIBA
Cordier *et al.* (1993); Viet Nam	Men	152	2	241	1	2.0	0.3–17	1989–92; hospital controls
Europe								
Stroffolini *et al.* (1992); Italy	Men and women	65	66	99	13	27	9.9–73	1990; all cases had cirrhosis and controls had non-hepatic chronic disease; adjusted for age, sex, hospital and HBV markers
	HCV antibody seropositive and HBsAg seropositive *versus* neither					77	3.8–1421	Adjusted for age, sex and hospital; 0.5 added to each entry; confirmation by RIBA
Zavitsanos *et al.* (1992); Greece	Men and women	181	13	446	1	10	4.2–26.0	1976–84; same subjects as Tzonou *et al.* (1991); unadjusted

[a]Cornfield limits

Table 10. Separate effects of HBV and HCV on risk for hepatocellular carcinoma

Reference and location	HBsAg seronegative HCV Ab seronegative		HBsAg seronegative HCV Ab seropositive			HBsAg seropositive HCV Ab seronegative			HBsAg seropositive HCV Ab seropositive		
	Cases	Controls	Cases	Controls	OR	Cases	Controls	OR	Cases	Controls	OR
First-generation tests											
Yu *et al.* (1990); USA	24	110	5	5	4.8	12	13	4.4	10	0	∞
Kaklamani *et al.* (1991); Greece	71	373	29	29	[5.3]	42	29	[7.6]	43	1	[226]
Yu *et al.* (1991)[a]; China	12	104	5	2	16	101	21	22	9	0	∞
Chuang *et al.* (1992)[a]; China	16	267	13	8	27	87	104	14	12	5	40
Simonetti *et al.* (1992); Italy	46	197	133	11	[52]	15	4	[16]	18	0	[∞]
Di Bisceglie et al. (1991a); USA	80	56	12	2	[7.2]	6	0	[∞]	1	0	[∞]
Xu *et al.* (1990); China	11	46	1	0	∞	35	4	[37]	3	0	∞
Second-generation tests											
Stroffolini *et al.* (1992); Italy	11	80	38	13	[21]	11	6	[13]	5	0	[∞]
Cordier *et al.* (1993); Viet Nam	8	194	3	2	38	138	44	[76]	0	0	-
Tanaka *et al.* (1991); Japan	27	390	45	12	[54]	18	8	[33]	1	0	[∞]
Coursaget *et al.* (1992); Senegal	23	82	4	0	[∞]	20	50	[1.4]	2	2	[3.6]
Dazza *et al.* (1993); Mozambique	52	163	8	4	1.4	115	27	[13]	3	0	[∞]

[a]Partially overlapping

hepatic neoplasms interspersed with areas of haemorrhage, necrosis and fibrosis; a smaller tumour (4 x 4 cm) was also seen. Frozen sections of liver stained with a monoclonal antibody against non-A, non-B-infected hepatocytes revealed positive immunostaining, but no specific staining for HBV surface or core antigens was observed. Histological examination of liver tumours revealed trabecular, well-differentiated HCC. The adjacent liver tissue contained hyperplastic nodules and severely dysplastic hepatocytes, as well as chronic hepatitis, characterized by portal inflammation and bile duct proliferation (Linke *et al.*, 1987; Muchmore *et al.*, 1988). [HCV markers were not evaluated.]

4. Other Relevant Data

4.1 Pathology

The putative association between HCV and HCC is based primarily on large epidemiological and serological reviews and not on histological studies of the evolution of carcinoma. The course of HCV includes acute viral hepatitis (usually subclinical) with transition to chronic hepatitis and cirrhosis and to HCC.

4.1.1 *Acute infection*

Many of the histological features of acute viral hepatitis are common to all four hepatitis viruses (HBV, HCV and hepatitis A and D viruses), and hepatic reactions are similar, so that histological features do not distinguish a specific agent. Some patients with acute hepatitis due to HCV, however, have a milder inflammatory reaction than those with disease due to HBV or hepatitis A virus, and this reaction may resemble infectious mononucleosis. The similarity to mononucleosis is due to sinusoidal inflammatory proliferation and portal lymphoid hyperplasia, without hepatocellular cytopathic change. In follow-up biopsy samples from patients with post-transfusion hepatitis, the hepatocellular changes may be very mild, and a diagnosis of acute viral hepatitis is based on clinical and histological correlation. The histological changes of acute viral hepatitis due to HCV are often mild and include the same inflammatory and hepatocellular degenerative changes seen in acute viral hepatitis due to HBV. Most observations of histopathological changes in acute viral hepatitis were restricted to clinically apparent, icteric cases, which constitute a minority of infections in a population. The majority of cases are therefore not confirmed histologically (Alter, 1990).

4.1.2 *Chronic infection*

The transition of acute viral hepatitis due to HCV to a progressive form of chronic hepatitis is usually gradual and not easily recognized by clinical or histological criteria. The same histopathological terminology for chronic hepatitis used for HBV has been widely applied to chronic hepatitis due to HCV. In addition, chronic hepatitis due to HCV often has some features not common to HBV, which include: portal lymphoid hyperplasia with germinal follicles, hepatocellular fatty change, bile-duct damage and multinucleated giant cells (Lefkowitch & Apfelbaum, 1989; Lefkowitch *et al.*, 1993). All of these features cannot

be diagnosed in a single case. In a follow-up study of chronic post-transfusion non-A, non-B hepatitis, 82% of cases were related to HCV, and a spectrum of hepatic lesions was described, which included chronic active hepatitis and cirrhosis; a few patients had minimal histological changes (Di Bisceglie *et al.*, 1991b). In another series of chronic non-A, non-B hepatitis patients followed for a longer period (3–20 years; mean, 8 years), 75% were due to HCV. Multiple biopsy samples from 24 patients with chronic post-transfusion non-A, non-B hepatitis revealed a spectrum of chronic hepatitis, ranging from mild chronic persistent hepatitis (55%) to chronic active hepatitis with cirrhosis (16%); the other cases were sporadic non-A, non-B hepatitis (Hopf *et al.*, 1990). Kiyosawa *et al.* (1990a) examined 231 patients with chronic non-A, non-B hepatitis and correlated the histological features with time after transfusion. The mean time since transfusion was 10 years for 96 patients with chronic hepatitis, 21.2 years for 81 with cirrhosis and and 29 years for 54 with HCC. Histological and serological data in several of these reports showed slow sequential progression of chronic hepatitis to cirrhosis and HCC.

4.1.3 *Cirrhosis and hepatocellular carcinoma*

The pathogenesis of cirrhosis is described in detail in the monograph on HBV (pp. 114–115). In most of the surveys of chronic hepatitis C, HCC developed in cirrhotic patients and not only in those with early chronic active hepatitis. This point has not been widely studied, but the situation appears to be different from that for HBV.

The transition of regenerative nodules to HCC is also described above. Ferrell *et al.* (1992b) examined 110 sequential explant livers with cirrhosis: 19 livers had 40 distinctive nodules measuring 0.8–3.5 cm. After careful histological examination, 28 were categorized as macroregenerative nodules and 12 as small HCCs. Thirty of the 110 cirrhotic livers were from patients with antibodies to HCV, which was associated with a greater risk for distinctive nodule formation (47%) than for all causes of cirrhosis in the series. Furthermore, patients with cirrhosis who were seropositive for HCV markers had an increased risk for incidental HCC; thus, four of the eight patients with HCC were HCV seropositive.

4.2 Molecular biology

The presence of minus-strand HCV RNA (implying replication) has been demonstrated in HCC and non-tumorous tissue (Gerber *et al.*, 1992; Gerber, 1993). There is at present no experimental evidence that HCV sequences are integrated into the host cell genome or for an HCV coded *trans*-activating protein.

Comparison of the nucleotide and predicted encoded amino acid sequences of HCV isolates in chronically infected chimpanzees suggest that the HCV genome is susceptible to mutations, as has been observed frequently for other RNA viruses (Okamoto *et al.*, 1992b). Genomic mutations of HCV in the course of chronic infections might promote persistence of the virus and its pathogenicity. In chimpanzees inoculated sequentially with different HCV strains derived from five unrelated patients with transfusion-associated non-A, non-B hepatitis, infection did not elicit protective immunity against reinfection with homologous or heterologous strains (Farci *et al.*, 1992b).

4.3 Other observations relevant to possible mechanisms of action of HCV in carcinogenesis

Chimpanzees were originally the primary model for studying the biology of HCV. In eight chimpanzees infected experimentally with HCV, three patterns of hepatitis C viraemia were seen: (i) acute resolving hepatitis with transient appearance of HCV RNA, (ii) chronic hepatitis with persistent HCV RNA and (iii) chronic hepatitis with intermittent appearance of HCV RNA (Abe *et al.*, 1992).

Analysis of serial clinical specimens obtained from chimpanzees infected experimentally with HCV revealed a relatively uniform relationship between peak expression of tissue markers of infection, appearance of HCV RNA in serum and elevated levels of serum ALT. Since the cytoplasmic antigen becomes detectable in infected chimpanzees within one week after inoculation, it was speculated that replication of HCV occurred very early in the incubation phase of hepatitis (Shimizu *et al.*, 1990). In another study (Shindo *et al.*, 1992b), the levels of HCV RNA in both serum and liver samples from champanzees paralleled disease activity, as measured by serum ALT levels. Serum ALT rose two days after inoculation, fell to normal levels between 10 and 14 days and rose to peak values at eight weeks. Serum HCV RNA became detectable by cDNA PCR three days after inoculation, persisted during the increase in serum ALT and was maximal at seven weeks. The first antibodies to appear, at nine weeks, were anti-C33c, directed against epitopes coded by the non-structural 3′ region of the genome; anti-C100-3 appeared at 12 weeks and antibody to 5-1-1 antigen shortly thereafter. Approximately 30–75% of the infected chimpanzees developed chronic infections, detected by the presence of genomic and anti-genomic viral sequences in the liver (Abe *et al.*, 1992; Farci *et al.*, 1992a; Hilfenhaus *et al.*, 1992). In the animals that developed chronic infection, both HCV RNA and anti-C100 (as detected in a first-generation test) remained persistently detectable.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Hepatitis C virus (HCV) is an RNA virus that is distantly related to flaviviruses and pestiviruses. The viral genome is a linear positive-strand RNA molecule about 9.4 kilobases long. It has a single, large open reading frame which encodes a polypeptide precursor of about 3000 amino acids. Viral isolates from different geographical regions display significant genetic diversity; in addition, different HCV genotypes can coexist in infected individuals. HCV infection has been detected only in humans, but the virus can be transmitted experimentally to chimpanzees.

HCV infection can be detected in serum by measuring antibody against HCV or directly measuring HCV RNA in blood. Seropositivity to HCV antibody correlates well with HCV infectivity; second-generation tests involving multiple antigenic epitopes show higher sensitivity and specificity than earlier methods. Measurement of HCV RNA is the most sensitive of the currently available tests and allows specific diagnosis in the early acute phase of infection. Replication of HCV in cell culture has been reported. Virus particles and identification of protective or neutralizing antibodies have not yet been demonstrated.

HCV causes most cases of non-A, non-B, post-transfusion hepatitis and a variable proportion of non-transfusion-associated, community-acquired non-A, non-B hepatitis. In most populations of the world, 0.5–2% of individuals have serological evidence of past or current infection. In most countries, prevalence increase with age in adult life and is approximately equal in men and women. A high prevalence of seropositivity is found in people with blood clotting disorders, in those on renal dialysis and in intravenous drug users. Transmission is mostly parenteral, although the route of infection in a significant proportion of cases of community-acquired infection is unknown. Both sexual and perinatal transmission occur.

The clinical course of acute HCV infection is mostly asymptomatic, but acute infection leads to chronic liver disease in about 50% of symptomatic patients and to liver cirrhosis in about 20% of those with chronic liver disease. Advanced liver disease and its complications may be the first clinical evidence of chronic HCV infection. Immunoprophylaxis for HCV infection is not available.

5.2 Human carcinogenicity data

Infection with HCV, as indicated by the presence of antibodies to HCV in serum, appeared to be associated with an increased risk for hepatocellular carcinoma in two cohorts of patients with chronic liver disease and in one cohort of the general population.

Over 20 case–control studies have evaluated the association between hepatocellular carcinoma and seropositivity for HCV antibodies, measured by either first- or second-generation tests. Odds ratio estimates ranging from 1.3 to 134 were observed in 17 studies in which first-generation tests were used and were significant in 15 of the studies. In six studies in which second-generation tests were used, the estimated odds ratios ranged from 1.1 to 52 and were significant in three of the studies.

In all 11 studies in which it could be evaluated, the risk for hepatocellular carcinoma was greater in subjects who were seropositive for antibodies to HCV and seronegative for hepatitis B surface antigen than in subjects seronegative for both. In the few studies in which the analysis took into account possible confounding of the effects of HCV by other risk factors for hepatocellular carcinoma, such as smoking and alcohol consumption, the association was not materially altered.

5.3 Animal carcinogenicity data

A single chimpanzee inoculated with serum from a human patient with non-A, non-B hepatitis developed chronic hepatitis; hepatocellular carcinoma occurred seven years after the first inoculation. Markers of hepatitis B viral infection were not found; the results of tests for HCV were not reported.

5.4 Other relevant data

HCV can replicate in hepatocellular carcinoma cells, but there is no evidence that DNA sequences are integrated into the host genome. Virtually all cases of HCV-related hepatocellular carcinoma occur in the presence of cirrhosis or significant chronic hepatitis.

5.5 Evaluation[1]

There is *sufficient evidence* in humans for the carcinogenicity of chronic infection with hepatitis C virus.

There is *inadequate evidence* in experimental animals for the carcinogenicity of hepatitis C virus.

Overall evaluation

Chronic infection with hepatitis C virus *is carcinogenic to humans (Group 1).*

6. References

Aach, R.D., Stevens, C.E., Hollinger, F.B., Mosley, J.W., Peterson, D.A., Taylor, P.E., Johnson, R.G., Barbosa, L.H. & Nemo, G.J. (1991) Hepatitis C virus infection in post-transfusion hepatitis. An analysis with first- and second-generation assays. *New Engl. J. Med.*, **325**, 1325–1329

Abb, J. (1991) Prevalence of hepatitis C virus antibodies in hospital personnel. *Zbl. Bakt.*, **274**, 543–547

Abe, K., Inchauspe, G., Shikata, T. & Prince, A.M. (1992) Three different patterns of hepatitis C virus infection in chimpanzees. *Hepatology*, **15**, 690–695

Aceti, A., Taliani, G., Bruni, R., Di Mauro, G. & Celestino, D. (1992) Hepatitis C virus antibody in Swaziland. *Acta trop.*, **51**, 159–161

Agnello, V., Chung, R.T. & Kaplan, L.M. (1992) A role for hepatitis C virus infection in type II cryoglobulinemia. *New Engl. J. Med.*, **327**, 1490–1495

Akahane, Y., Aikawa, T., Sugai, Y., Tsuda, F., Okamoto, H. & Mishiro, S. (1992) Transmission of HCV between spouses (Letter to the Editor). *Lancet*, **339**, 1059–1060

Akyol, G., Dash, S., Shieh, Y.S.C., Malter, J.S. & Gerber, M.A. (1992) Detection of hepatitis C virus RNA sequences by polymerase chain reaction in fixed liver tissue. *Mod. Pathol.*, **5**, 501–504

Albano, A., Pianetti, A., Biffi, M.R., Bruscolini, F., Baffone, W., Albano, V. & Salvaggio, A. (1992) Prevalence of anti-HCV in subjects of various age groups. *Eur. J. Epidemiol.*, **8**, 309–311

Alberti, A. (1991) Diagnosis of hepatitis C. Facts and perspectives. *J. Hepatol.*, **12**, 279–282

Alberti, A., Morsica, G., Chemello, L., Cavalletto, D., Noventa, F., Pontisso, P. & Ruol, A. (1992) Hepatitis C viraemia and liver disease in symptom-free individuals with anti-HCV. *Lancet*, **340**, 697–698

Al Karawi, M.A., Shariq, S., El Shiekh Mohamed, A.R., Saeed, A.A. & Mohamed Ahmed, A.M. (1992) Hepatitis C virus infection in chronic liver disease and hepatocellular carcinoma in Saudi Arabia. *J. Gastroenterol. Hepatol.*, **7**, 237–239

Al-Nakib, B., Koshy, A., Kaloui, M., Al-Ramahi, S., Al-Mafti, S., Radhakrishnan, S. & Al-Natib, W. (1992) Hepatitis C virus antibody in Kuwait (Letter to the Editor). *Vox sang.*, **63**, 75–76

Al Nasser, M.N., Al Mugeiren, M.A., Assuhaimi, S.A., Obineche, E., Onwabalili, J. & Ramia, S. (1992) Seropositivity to hepatitis C virus in Saudi haemodialysis patients. *Vox sang.*, **62**, 94–97

Alter, H.J. (1990) The hepatitis C virus and its relationship to the clinical spectrum of NANB hepatitis. *J. Gastroenterol. Hepatol.*, **Suppl. 1**, 78–94

[1]For definition of the italicized terms, see Preamble, pp. 30–34.

Alter, H.J., Purcell, R.H., Holland, P.V. & Popper, H. (1978) Transmissible agent in non-A, non-B hepatitis. *Lancet*, **i**, 459–463

Alter, M.J., Gerety, R.J., Smallwood, L.A., Sampliner, R.E., Tabor, E., Deinhardt, F., Frösner, G. & Matanoski, G.M. (1982) Sporadic non-A, non-B hepatitis: frequency and epidemiology in an urban US population. *J. infect. Dis.*, **145**, 886–893

Alter, H.J., Purcell, R.H., Shih, J.W., Melpolder, J.C., Houghton, M., Choo, Q.-L. & Kuo, G. (1989) Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis. *New Engl. J. Med.*, **321**, 1494–1500

Alter, M.J., Coleman, P.J., Alexander, W.J., Kramer, E., Miller, J.K., Mandel, E., Hadler, S.C. & Margolis, H.S. (1989) Importance of heterosexual activity in the transmission of hepatitis B and non-A, non-B hepatitis. *J. Am. med. Assoc.*, **262**, 1201–1205

Alter, M.J., Margolis, H.S., Krawczynski, K., Judson, F.N., Mares, A., Alexander, W.J., Hu, P.Y., Miller, J.K., Gerber, M.A., Sampliner, R.E., Meeks, E.L. & Beach, M.J. (1992) The natural history of community-acquired hepatitis C in the United States. *New Engl. J. Med.*, **327**, 1899–1905

Amitrano, L., Ascione, A., Canestrini, C., D'Agostino, S., Iaccarino, L., Vacca, C. & Gigliotti, T. (1990) Prevalence of antibody to hepatitis C virus (anti-HCV) in chronic liver diseases (CLD) in southern Italy. *Ital. J. Gastroenterol.*, **22**, 16–18

Anand, C.M., Fonseca, K., Walle, R.P., Powell, S. & Williams, M. (1992) Antibody to hepatitis C virus in selected groups of a Canadian urban population. *Int. J. Epidemiol.*, **21**, 142–145

Anon. (1975) Non-A, non-B? (Editorial) *Lancet*, **ii**, 64–65

Archer, G.T., Buring, M.L., Clark, B., Ismay, S.L., Kenrick, K.G., Pususothaman, K., Kaldor, J.M., Bolton, W.V. & Wylie, B.R. (1992) Prevalence of hepatitis C virus antibodies in Sydney blood donors. *Med. J. Aust.*, **157**, 225–227

Aussel, L., Denis, F., Ranger, S., Martin, P., Caillaud, M., Alain, J., Baudet, J. & Tabaste, J.L. (1991) Search for antibodies to hepatitis C virus in pregnant foreign women living in France. *Pathol. Biol.*, **39**, 991–996 (in French)

Bahakim, H.M., Bakir, T.M.F, Arif, M. & Ramia, S. (1991) Response to comment by Bernvil *et al.* (Letter to the Editor). *Vox sang.*, **61**, 280

Baur, M., Hay, U., Novacek, G., Dittrich, C. & Ferenci, P. (1992) Prevalence of antibodies to hepatitis C virus in patients with hepatocellular carcinoma in Austria. *Arch. Virol.*, **Suppl. 4**, 76–80

Beach, M.J., Meeks, E.L., Mimms, L.T., Vallari, D., DuCharme, L., Spelbring, J., Taskar, S., Schleicher, J.B., Krawczynski, K. & Bradley, D.W. (1992) Temporal relationships of hepatitis C virus RNA and antibody responses following experimental infection of chimpanzees. *J. med. Virol.*, **36**, 226–237

Bell, J., Batey, R.G., Farrell, G.C., Crewe, E.B., Cunningham, A.L. & Byth, K. (1990) Hepatitis C virus in intravenous drug users. *Med. J. Aust.*, **153**, 274–276

Belli, L.S., Alberti, A., Rondinara, G.F., De Carlis, L., Romani, F., Ideo, G. & Belli, L. (1993) Recurrent HCV hepatitis after liver transplantation (Letter to the Editor). *Lancet*, **341**, 378–379

Benvegnù, L., Cavalletto, D., Ruvoletto, M.G. & Alberti, A. (1991) Specificity and patterns of antibodies to hepatitis C virus in hepatocellular carcinoma (Letter to the Editor). *Hepatology*, **14**, 959

Bernvil, S.S., Andrews, V.J. & Kariem, A.A. (1991) Hepatitis C antibody prevalence in Saudi Arabian blood donor population. *Ann. Saudi Med.*, **11**, 563–567

Black, M. & Peters, M. (1992) Alpha-interferon treatment of chronic hepatitis C: need for accurate diagnosis in selecting patients. *Ann. intern. Med.*, **116**, 86–88

Blackmore, T.K., Maddocks, P., Stace, N.H. & Hatfield, P. (1992) Prevalence of antibodies to hepatitis C virus in patients receiving renal replacement therapy, and in the staff caring for them. *Aust. N.Z. J. Med.*, **22**, 353–357

van Bohman, R., Stettler, R.W., Little, B.B., Wendel, G.D., Sutor, L.J. & Cunningham, F.G. (1992) Seroprevalence and risk factors for hepatitis C virus antibody in pregnant women. *Obstet. Gynaecol.*, **80**, 609–613

Boonmar, S., Pojanagaroon, B., Watanabe, Y., Tanaka, Y., Saito, I. & Miyamura, T. (1990) Prevalence of hepatitis C virus antibody among healthy blood donors and non-A, non-B hepatitis patients in Thailand. *Jpn. J. med. Sci. Biol.*, **43**, 29–36

Boyer, N., Marcellin, P., Degott, C., Degos, F., Saimot, A.G., Erlinger, S., Benhamou, J.P. & the 'Comité des Anti-viraux' (1992) Recombinant interferon-alpha for chronic hepatitis C in patients positive for antibody to human immunodeficiency virus. *J. infect. Dis.*, **165**, 723–726

Bradley, D.W., Cook, E.H., Maynard, J.E., McCaustland, K.A., Ebert, J.W., Dolana, G.H., Petzel, R.A., Kantor, R.J., Heilbrunn, A., Fields, H.A. & Murphy, B.L. (1979) Experimental infection of chimpanzees with anti-hemophilic (factor VIII) materials: recovery of virus-like particles associated with with non-A, non-B hepatitis. *J. med. Virol.*, **3**, 253–269

Bradley, D.W., Krawczynski, K., Ebert, J.W., McCaustland, K.A., Choo, Q.-L., Houghton, M. & Kuo, G. (1990) Parenterally transmitted non-A, non-B hepatitis: virus-specific antibody response patterns in hepatitis C virus-infected chimpanzees. *Gastroenterology*, **99**, 1054–1060

Bresters, D., Cuypers, H.T.M., Reesink, H.W., Chamuleau, R.A.F.M., Schipper, M.E.I., Boeser-Nunnink, B.D.M., Lelie, P.N. & Jansen, P.L.M. (1992) Detection of hepatitis C viral RNA sequences in fresh and paraffin-embedded liver biopsy specimens of non-A, non-B hepatitis patients. *J. Hepatol.*, **15**, 391–395

Brettler, D.B., Alter, H.J., Dienstag, J.L., Forsberg, A.D. & Levine, P.H. (1990) Prevalence of hepatitis C virus antibody in a cohort of hemophilia patients. *Blood*, **76**, 254–256

Brugnano, R., Francisci, D., Quintaliani, G., Gaburri, M., Nori, G., Verdura, C., Giombini, L. & Buoncristiani, U. (1992) Antibodies against hepatitis C virus in hemodialysis patients in the central Italian region of Umbria: evaluation of risk factors. *Nephron*, **61**, 263–265

Bruix, J., Calvet, X., Costa, J., Ventura, M., Bruguera, M., Castillo, R., Barrera, J.M., Ercilla, G., Sanchez-Tapias, J.M., Vall, M., Bru, C. & Rodes, J. (1989) Prevalence of antibodies to hepatitis C virus in Spanish patients with hepatocellular carcinoma and hepatic cirrhosis. *Lancet*, **ii**, 1004–1006

Bukh, J., Miller, R.H., Kew, M.C. & Purcell, R.H. (1993) Hepatitis C virus RNA in southern African blacks with hepatocellular carcinoma. *Proc. natl Acad. Sci. USA*, **90**, 1848–1851

Calabrese, G., Vagelli, G., Guaschino, R. & Gonella, M. (1991) Transmission of anti-HCV within the household of haemodialysis patients (Letter to the Editor). *Lancet*, **ii**, 1466

Caporaso, N., Romano, M., Marmo, R., de Sio, I., Morisco, F., Minerva, A. & Coltorti, M. (1991) Hepatitis C virus infection is an additive risk factor for development of hepatocellular carcinoma in patients with cirrhosis. *J. Hepatol.*, **12**, 367–371

Carreño, V., Tapia, L., Ryff, J.-C., Quiroga, J.A. & Castillo, I. (1992) Treatment of chronic hepatitis C by continuous subcutaneous infusion of interferon-alpha. *J. med. Virol.*, **37**, 215–219

Caspari, G., Beyer, J., Richter, K., Gerlich, W.H. & Schmitt, H. (1991) Prevalence of antibodies to recombinant hepatitis C virus protein C100-3 and of elevated transaminase levels in blood donors from northern Germany. *Med. Microbiol. Immunol.*, **180**, 261–272

Cha, T.-A., Beall, E., Irvine, B., Kolberg, J., Chien, D., Kuo, G. & Urdea, M.S. (1992) At least five related, but distinct, hepatitis C viral genotypes exist. *Proc. natl Acad. Sci. USA*, **89**, 7144–7148

Chan, G.C.B., Lim, W. & Yeoh, E.K. (1992) Prevalence of hepatitis C infection in Hong Kong. *J. Gastroenterol. Hepatol.*, **7**, 117–120

Chan, S.-W., McOmish, F., Holmes, E.C., Dow, B., Peutherer, J.F., Follett, E., Yap, P.L. & Simmonds, P. (1992) Analysis of a new hepatitis C virus type and its phylogenetic relationship to existing variants. *J. gen. Virol.*, **73**, 1131–1141

Chaudhary, R.K., Perry, E., Hicks, F., MacLean, C. & Morbey, M. (1992) Hepatitis B and C infection in an institution of the developmentally handicapped (Letter to the Editor). *New Engl. J. Med.*, **327**, 1953

Chen, P.-J., Lin, M.-H., Tai, K.-F., Liu, P.-C., Lin, C.-J. & Chen, D.-S. (1992) The Taiwanese hepatitis C virus genome: sequence determination and mapping of the 5′ termini of viral genomic and antigenomic RNA. *Virology*, **188**, 102–113

Chiba, J., Ohba, H., Matsuura, Y., Watanabe, Y., Katayama, T., Kikuchi, S., Saito, I. & Miyamura, T. (1991) Serodiagnosis of hepatitis C virus (HCV) infection with an HCV core protein molecularly expressed by a recombinant baculovirus. *Proc. natl Acad. Sci. USA*, **88**, 4641–4645

Chien, D.Y., Choo, Q.-L., Tabrizi, A., Kuo, C., McFarland, J., Berger, K., Lee, C., Shuster, J.R., Nguyen, T., Moyer, D.L., Tong, M., Furuta, S., Omata, M., Tegtmeier, G., Alter, H., Schiff, E., Jeffers, L., Houghton, M. & Kuo, G. (1992) Diagnosis of hepatitis C virus (HCV) infection using an immunodominant chimeric polyprotein to capture circulating antibodies: reevaluation of the role of HCV in liver disease. *Proc. natl Acad. Sci. USA*, **89**, 10011–10015

Choo, Q.-L., Kuo, G., Weiner, A.J., Overby, L.R., Bradley, D.W. & Houghton, M. (1989) Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science*, **244**, 359–362

Choo, Q.-L., Richman, K.H., Han, J.H., Berger, K., Lee, C., Dong, C., Gallegos, C., Coit, D., Medina-Selby, A., Barr, P.J., Weiner, A.J., Bradley, D.W., Kuo, G. & Houghton, M. (1991) Genetic organization and diversity of the hepatitis C virus. *Proc. natl Acad. Sci. USA*, **88**, 2451–2455

Chuang, W.-L., Chang, W.-Y., Lu, S.-N., Su, W.-P., Lin, Z.-Y., Chen, S.-C., Hsieh, M.-Y., Wang, L.-Y., You, S.-L. & Chen, C.-J. (1992) The role of hepatitis B and C viruses in hepatocellular carcinoma in a hepatitis B endemic area: a case–control study. *Cancer*, **69**, 2052–2054

Claeys, H., Volckaerts, A., De Beenhouwer, H. & Vermylen, C. (1992) Association of hepatitis C virus carrier state with the occurrence of hepatitis C virus core antibodies. *J. med. Virol.*, **36**, 259–264

Colombo, M., Rumi, M.G., Gringeri, A. & Mannucci, P.M. (1988) Etiology and outcome of chronic viral hepatitis in multitranfused hemophilic patients. In: Gentilini, P. & Dianzani, M.U., eds, *Pathophysiology of the Liver*, Amsterdam, Elsevier, pp. 73–79

Colombo, M., Kuo, G., Choo, Q.L., Donato, M.F., Del Ninno, E., Tommasini, M.A., Dioguardi, N. & Houghton, M. (1989) Prevalence of antibodies to hepatitis C virus in Italian patients with hepatocellular carcinoma. *Lancet*, **ii**, 1006–1008

Cordier, S., Thuy, Le Thi Bich, Verger, P., Bard, D., Dai, Le Cao, Larouze, B., Dazza, M.C., Quinh, Hoang Trong & Abenhaim, L. (1993) Risk factors for hepatocellular carcinoma in Vietnam. *Int. J. Epidemiol.*, **55**, 196–201

Corona, R., Prignano, G., Mele, A., Gentili, G., Caprilli, F., Franco, E., Ferrigno, L., Giglio, A., Titti, F., Bruno, C., Verani, P. & Pasquini, P. (1991) Heterosexual and homosexual transmission of hepatitis C virus: relation with hepatitis B virus and human immunodeficiency virus type 1. *Epidemiol. Infect.*, **107**, 667–672

Coursaget, P., Bourdil, C., Kastally, R., Yvonnet, B., Rampanarivo, Z., Chiron, J.-P., Bao, O., Diop-Mar, I., Perrin, J. & Ntarème, F. (1990) Prevalence of hepatitis C virus infection in Africa: anti-HCV antibodies in the general population and in patients suffering from cirrhosis or primary liver cancer. *Res. Virol.*, **141**, 449–454

Coursaget, P., Leboulleux, D., Le Cann, P., Bao, O. & Coll-Seck, A.M. (1992) Hepatitis C virus infection in cirrhosis and primary hepatocellular carcinoma in Senegal. *Trans. R. Soc. trop. Med. Hyg.*, **86**, 552–553

Czaja, A.J. (1992) Chronic hepatitis C virus infection—a disease in waiting? *New Engl. J. Med.*, **327**, 1949–1950

Dal-Ré, R., Aguilar, L. & Coronel, P. (1991) Current prevalence of hepatitis B, A and C in a healthy Spanish population. A seroepidemiological study. *Infection*, **19**, 409–413

Davis, G.L. (1989) Interferon treatment of viral hepatitis in immunocompromised patients. *Semin. Liver Dis.*, **9**, 267–272

Davis, G.L., Balart, L.A., Schiff, E.R., Lindsay, K., Bodenheimer, H.C., Jr, Perrillo, R.P., Carey, W., Jacobson, I.M., Payne, J., Dienstag, J.L., Van Thiel, D.H., Tamburro, C., Lefkowitch, J., Albrecht, J., Meschievitz, C., Ortego, T.J., Gibas, A. & the Hepatitis Interventional Therapy Group (1989) Treatment of chronic hepatitis C with recombinant interferon alfa. A multicenter randomized, controlled trial. *New Engl. J. Med.*, **321**, 1501–1506

Dazza, M.-C., Valdemar Meneses, L., Girard, P.-M., Astagneau, P., Villaroel, C., Delaporte, E. & Larouze, B. (1993) Absence of a relationship between antibodies to hepatitis C virus and hepatocellular carcinoma in Mozambique. *J. trop. Med. Hyg.*, **48**, 237–242

De Luca, M., Ascione, A., Canestrini, C., Di Costanzo, G.G., Galeota Lanza, A., Guardascione, M.A., Gallo, M.L., Perrotti, G.P., Samaritani, C.M., Utech, W., Iaccarino, L., Vacca, C., Pietroluongo, E., Schiano, S., Tarsitano, P. & Zarone, A. (1991) Prevalence of antibody to hepatitis C virus in hospital personnel. *Minerva Gastroenterol. Dietol.*, **37**, 141–149 (in Italian)

De Luca, M., Ascione, A., Vacca, C. & Zarone, A. (1992) Are health-care workers really at risk of HCV infection? (Letter to the Editor) *Lancet*, **339**, 1364–1365

Deny, P., Bonacorsi, S., Guillevin, L. & Quint, L. (1992) Association between hepatitis C virus and polyarteritis nodosa (Letter to the Editor). *Clin. exp. Rheumatol.*, **10**, 319

Develoux, M., Meynard, D. & Delaporte, E. (1992) Low rate of hepatitis C virus antibodies in blood donors and pregnant women from Niger. *Trans. R. Soc. trop. Med. Hyg*, **86**, 553

Diamantis, I.D., McGandy, C.E., Pult, I., Widmann, J.-J., Gudat, F. & Bianchi L. (1992) Detection of hepatitis C virus RNA in liver needle biopsies by polymerase chain reaction. *J. Hepatol.*, **14**, 405–406

Di Bisceglie, A.M., Martin, P., Kassianides, C., Lisker-Melman, M., Murray, L., Waggoner, J., Goodman, Z., Banks, S.M. & Hoofnagle, J.H. (1989) Recombinant interferon alfa therapy for chronic hepatitis C. A randomized, double-blind, placebo-controlled trial. *New Engl. J. Med.*, **321**, 1506–1510

Di Bisceglie, A.M., Order, S.E., Klein, J.L., Waggoner, J.G., Sjogren, M.H., Kuo, G., Houghton, M., Choo, Q.-L. & Hoofnagle, J.H. (1991a) The role of chronic viral hepatitis in hepatocellular carcinoma in the United States. *Am. J. Gastroenterol.*, **86**, 335–338

Di Bisceglie, A.M., Goodman, Z.D., Ishak, K.G., Hoofnagle, J.H., Melpolder, J.J. & Alter, H.J. (1991b) Long-term clinical and histopathological follow-up of chronic posttransfusion hepatitis. *Hepatology*, **14**, 969–974

Di Bisceglie, A.M., Shindo, M., Fong, T.-L., Fried, M.W., Swain, M.G., Bergasa, N.V., Axiotis, C.A., Waggoner, J.G., Park, Y. & Hoofnagle, J.H. (1992) A pilot study of ribavirin therapy for chronic hepatitis C. *Hepatology*, **16**, 649–654

Donahue, J.G., Nelson, K.E., Muñoz, A., Vlahov, D., Rennie, L.L., Taylor, E.L., Saal, A.J., Cohn, S., Odaka, N.J. & Farzadegan, H. (1991) Antibody to hepatitis C virus among cardiac surgery patients, homosexual men and intravenous drug users in Baltimore, Maryland. *Am. J. Epidemiol.*, **134**, 1206–1211

El-Ashmawy, L., Hassanein, T., Gavaler, J.S. & Van Thiel, D.H. (1992) Prevalence of hepatitis C virus antibody in a liver transplantation population. *Dig. Dis. Sci.*, **37**, 1110–1115

Enomoto, N., Takada, A., Nakao, T. & Date, T. (1990) There are two major types of hepatitis C virus in Japan. *Biochem. biophys. Res. Commun.*, **170**, 1021–1025

Esteban, J.I., Esteban, R., Viladomiu, L., López-Talavera, J.C., González, A., Hernández, J.M., Roget, M., Vargas, V., Genescà, J., Buti, M., Guardia, J., Houghton, M., Choo, Q.-L. & Kuo, G. (1989) Hepatitis C virus antibodies among risk groups in Spain. *Lancet*, **ii**, 294–297

Fabrizi, F., Di Filippo, S., Erba, G., Bacchini, G., Marcelli, D., Pontoriero, G., Crepaldi, M. & Locatelli, F. (1992) Hepatitis C virus infection and hepatic function in chronic hemodialysis patients (Letter to the Editor). *Nephron*, *61*, 119

Fairley, C.K., Leslie, D.E., Nicholson, S. & Gust, I.D. (1990) Epidemiology and hepatitis C virus in Victoria. *Med. J. Aust.*, **153**, 271–273

Farci, P., Alter, H.J., Wong, D., Miller, R.H., Shih, J.W., Jett, B. & Purcell, R.H. (1991) A long-term study of hepatitis C virus replication in non-A, non-B hepatitis. *New Engl. J. Med.*, **325**, 98–104

Farci, P., London, W.T., Wong, D.C., Dawson, G.J., Vallari, D.S., Engle, R. & Purcell, R.H. (1992a) The natural history of infection with hepatitis C virus (HCV) in chimpanzees: comparison of serologic responses measured with first- and second-generation assays and relationship to HCV viremia. *J. infect. Dis.*, **165**, 1006–1011

Farci, P., Alter, H.J., Govindarajan, S., Wong, D.C., Engle, R., Lesniewski, R.R., Mushahwar, I.K., Desai, S.M., Miller, R.H., Ogata, N. & Purcell, R.H. (1992b) Lack of protective immunity against reinfection with hepatitis C virus. *Science*, **258**, 135–140

Fargion, S., Piperno, A., Cappellini, M.D., Sampietro, M., Fracanzani, A.L., Romano, R., Caldarelli, R., Marcelli, R., Vecchi, L. & Fiorelli, G. (1992) Hepatitis C virus and porphyria cutanea tarda: evidence of a strong association. *Hepatology*, **16**, 1322–1326

Farinati, F., Fagiuoli, S., De Maria, N., Chiaramonte, M., Aneloni, V., Ongaro, S., Salvagnini, M. & Naccarato, R. (1992) Anti-HCV positive hepatocellular carcinoma in cirrhosis: prevalence, risk factors and clinical features. *J. Hepatol.*, **14**, 183–187

Féray, C., Samuel, D., Thiers, V., Gigou, M., Pichon, F., Bismuth, A., Reynes, M., Maisonneuve, P., Bismuth, H. & Bréchot, C. (1992) Reinfection of liver graft by hepatitis C virus after liver transplantation. *J. clin. Invest.*, **89**, 1361–1365

Féray, C., Gigou, M., Samuel, D., Reyes, G., Bernuau, J., Reynes, M., Bismuth, H. & Bréchot, C. (1993) Hepatitis C virus RNA and hepatitis B virus DNA in serum and liver of patients with fulminant hepatitis. *Gastroenterology*, **104**, 549–555

Ferrell, L.D., Wright, T.L., Roberts, J., Ascher, N. & Lake, J. (1992a) Hepatitis C viral infection in liver transplant recipients. *Hepatology*, **16**, 865–876

Ferrell, L.D., Wright, T., Lake, J., Roberts, J. & Ascher, N. (1992b) Incidence and diagnostic features of macroregenerative nodules vs. small hepatocellular carcinoma in cirrhotic livers. *Hepatology*, **16**, 1372–1381

Fong, T.-L., Shindo, M., Feinstone, S.M., Hoofnagle, J.H. & Di Bisceglie, A.M. (1991) Detection of replicative intermediates of hepatitis C viral RNA in liver and serum of patients with chronic hepatitis C. *J. clin. Invest.*, **88**, 1058–1060

Fried, M.W., Shindo, M., Fong, T.-L., Fox, P.C., Hoofnagle, J.H. & Di Bisceglie, A.M. (1992) Absence of hepatitis C viral RNA from saliva and semen of patients with chronic hepatitis C. *Gastroenterology*, **102**, 1306–1308

Fuchs, K., Motz, M., Schreier, E., Zachoval, R., Deinhardt, F. & Roggendorf, M. (1991) Characterization of nucleotide sequences from European hepatitis C virus isolates. *Gene*, **103**, 163–169

Fujiyama, S., Kawano, S., Sato, S., Tanaka, M., Goto, M., Taura, Y., Sato, T., Kawahara, T. & Mizuno, K. (1992) Prevalence of hepatitis C virus antibodies in hemodialysis patients and dialysis staff. *Hepato-gastroenterology*, **39**, 161–165

Garson, J.A., Brillanti, S., Ring, C., Perini, P., Miglioli, M. & Barbara, L. (1992a) Hepatitis C viraemia rebound after 'successful' interferon therapy in patients with chronic non-A, non-B hepatitis. *J. med. Virol.*, **37**, 210–214

Garson, J.A., Wicki, A.N., Ring, C.J.A., Joller, H., Zala, G., Schmid, M. & Buehler, H. (1992b) Detection of hepatitis C viraemia in Caucasian patients with hepatocellular carcinoma. *J. med. Virol.*, **38**, 152–156

Gasparini, V., Chiaramonte, M., Moschen, M.E., Fabris, P., Altinier, G., Majori, S., Campello, C. & Trivello, R. (1991) Hepatitis C virus infection in homosexual men: a seroepidemiological study in gay clubs in north-east Italy. *Eur. J. Epidemiol.*, **7**, 665–669

Gerber, M.A. (1993) Relation of hepatitis C virus to hepatocellular carcinoma. *J. Hepatol.*, **17** (Suppl. 3), S108–S111

Gerber, M.A., Shieh, Y.S.C., Shim, K.-S., Thung, S.N., Demetris, A.J., Schwartz, M., Akyol, G. & Dash, S. (1992) Short communication. Detection of replicative hepatitis C virus sequences in hepatocellular carcinoma. *Am. J. Pathol.*, **141**, 1271–1277

Girardi, E., Zaccarelli, M., Tossini, G., Puro, V., Narciso, P. & Visco, G. (1990) Short communication. Hepatitis C virus infection in intravenous drug users: prevalence and risk factors. *Scand. J. infect. Dis.*, **22**, 751–752

Goodrick, M.J., Anderson, N.A.B., Fraser, I.D., Rouse, A. & Pearson, V. (1992) History of previous drug misuse in HCV-positive blood donors (Letter to the Editor). *Lancet*, **339**, 502

Greenwood, B.M. & Whittle, H.C. (1981) *Immunology of Medicine in the Tropics. Current Topics in Immunology*, London, E. Arnold

Gutierrez, P., Orduña, A., Bratos, M.A., Eiros, J.M., Gonzalez, J.M., Almaraz, A., Caro-Patón, A. & Rodríguez-Torres, A. (1992) Prevalence of anti-hepatitis C virus antibodies in positive FTA-ABS non-drug abusing female prostitutes in Spain. *Sex. Transmitted Dis.*, January–February, 39–40

Haddad, J., Deny, P., Munz-Gotheil, C., Ambrosini, J.-C., Trinchet, J.-C., Pateron, D., Mal, F., Callard, P. & Beaugrand, M. (1992) Lymphocytic sialadenitis of Sjøgren's syndrome associated with chronic hepatitis C virus liver disease. *Lancet*, **339**, 321–323

Hagiwara, H., Hayashi, N., Mita, E., Hiramatsu, N., Ueda, K., Takehara, T., Yuki, N., Kasahara, A., Fusamoto, H. & Kamada, T. (1992) Detection of hepatitis C virus RNA in chronic non-A, non-B liver disease. *Gastroenterology*, **102**, 692–694

Hagiwara, H., Hayashi, N., Mita, E., Takehara, T., Kasahara, A., Fusamoto, H. & Kamada, T. (1993) Quantitative analysis of hepatitis C virus RNA in serum during interferon alfa therapy. *Gastroenterology*, **104**, 877–883

Han, J.H., Shyamala, V., Richman, K.H., Brauer, M.J., Irvine, B., Urdea, M.S., Tekamp-Olson, P., Kuo, G., Choo, Q.-L. & Houghton, M. (1991) Characterization of the terminal regions of hepatitis C viral RNA: identification of conserved sequences in the 5′ untranslated region and poly(A) tails at the 3′ end. *Proc. natl Acad. Sci. USA*, **88**, 1711–1715

Harada, S., Watanabe, Y., Takeuchi, K., Suzuki, T., Katayama, T., Takebe, Y., Saito, I. & Miyamura, T. (1991) Expression of processed core protein of hepatitis C virus in mammalian cells. *J. Virol.*, **65**, 3015–3021

Hasan, F., Jeffers, L.J., De Medina, M., Reddy, K.R., Parker, T., Schiff, E.R., Houghton, M., Choo, Q. & Kuo, G. (1990) Hepatitis C-associated hepatocellular carcinoma. *Hepatology*, **12**, 589–591

Hay, C.R.M., Preston, F.E., Triger, D.R. & Underwood, J.C.E. (1985) Progressive liver disease in haemophilia: an understated problem? *Lancet*, **i**, 1495–1498

Herbert, A.-M., Walker, D.M., Davies, K.J. & Bagg, J. (1992) Occupationally acquired hepatitis C virus infection (Letter to the Editor). *Lancet*, **339**, 305

Hijikata, M., Kato, N., Ootsuyama, Y., Nakagawa, M., Ohkoshi, S. & Shimotohno, K. (1991) Hypervariable regions in the putative glycoprotein of hepatitis C virus. *Biochem. biophys. Res. Commun.*, **175**, 220–228

Hilfenhaus, J., Krupka, U., Nowak, T., Cummins, L.B., Fuchs, K. & Roggendorf, M. (1992) Follow-up of hepatitis C virus infection in chimpanzees: determination of viraemia and specific humoral immune response. *J. gen. Virol.*, **73**, 1015–1019

Hiramatsu, N., Hayashi, N., Haruna, Y., Kasahara, A., Fusamoto, H., Mori, C., Fuke, I., Okayama, H. & Kamada, T. (1992) Immunohistochemical detection of hepatitis C virus-infected hepatocytes in chronic liver disease with monoclonal antibodies to core, envelope and NS3 regions of the hepatitis C virus genome. *Hepatology*, **16**, 306–311

van den Hoek, J.A.R., van Haastrecht, H.J.A., Goudsmith, J., de Wolf, F. & Coutinho, R.A. (1990) Prevalence, incidence and risk factors of hepatitis C virus infection among drug users in Amsterdam. *J. infect. Dis.*, **162**, 823–826

Hopf, U., Möller, B., Küther, D., Stemerowicz, R., Lobeck, H., Lüdtke-Handjery, A., Walter, E., Blum, H.E., Roggendorf, M. & Deinhardt, F. (1990) Long-term follow-up of posttransfusion and sporadic chronic hepatitis non-A, non-B and frequency of circulating antibodies to hepatitis C virus (HCV). *J. Hepatol.*, **10**, 69–76

Hosoda, K., Omata, M., Yokosuka, O., Kato, N. & Ohto M. (1992) Non-A, non-B chronic hepatitis is chronic hepatitis C: a sensitive assay for detection of hepatitis C virus RNA in the liver. *Hepatology*, **15**, 777–781

Houghton, M., Weiner, A., Han, J., Kuo, G. & Choo, Q.-L. (1991) Molecular biology of the hepatitis C viruses: implications for diagnosis, development and control of viral disease. *Hepatology*, **14**, 381–388

Hsieh, T.-T., Yao, D.-S., Sheen, I.-S., Liaw, Y.-F. & Pao, C.C. (1992) Hepatitis C virus in peripheral blood mononuclear cells. *Am. J. clin. Pathol.*, **98**, 392–396

Hyams, K.C., Phillips, I.A., Moran, A.Y., Tejada, A., Wignall, F.S. & Escamilla, J. (1992) Seroprevalence of hepatitis C antibody in Peru. *J. med. Virol.*, **37**, 127–131

Inchauspé, G., Zebedee, S., Lee, D.-H., Sugitani, M., Nasoff, M. & Prince, A.M. (1991) Genomic structure of the human prototype strain H of hepatitis C virus: comparison with American and Japanese isolates. *Proc. natl Acad. Sci. USA*, **88**, 10292–10296

Inoue, Y., Miyamura, T., Unayama, T., Takahashi, K. & Saito, I. (1991) Maternal transfer of HCV (Letter to the Editor). *Nature*, **353**, 609

Ito, S.-I., Ito, M., Cho, M.-J., Shimotohno, K. & Tajima, K. (1991) Massive sero-epidemiological survey of hepatitis C virus: clustering of carriers on the southwest coast of Tsushima, Japan. *Jpn. J. Cancer Res.*, **82**, 1–3

Japanese Red Cross Non-A, Non-B Hepatitis Research Group (1991) Effect of screening for hepatitis C virus antibody and hepatitis B virus core antibody on incidence of post-transfusion hepatitis. *Lancet*, **338**, 1040–1041

Jeng, J.-E. & Tsai, J.-F. (1991) Hepatitis C virus antibody in hepatocellular carcinoma in Taiwan. *J. med. Virol.*, **34**, 74-77

Jochen, A.B.B. (1992) Occupationally acquired hepatitis C virus infection (Letter to the Editor). *Lancet*, **339**, 304

Johnson, R.J., Gretch, D.R., Yamabe, H., Hart, J., Bacchi, C.E., Hartwell, P., Couser, W.G., Corey, L., Wener, M.H., Alpers, C.E. & Willson, R. (1993) Membranoproliferative glomerulonephritis associated with hepatitis C virus infection. *New Engl. J. Med.*, **328**, 465–470

Kaklamani, E., Trichopoulos, D., Tzonou, A., Zavitsanos, X., Koumantaki, Y., Hatzakis, A., Hsieh, C.C. & Hatziyannis, S. (1991) Hepatitis B and C viruses and their interaction in the origin of hepatocellular carcinoma. *J. Am. med. Assoc.*, **265**, 1974–1976

Kaldor, J.M., Archer, G.T., Buring, M.L., Ismay, S.L., Kenrick, K.G., Lien, A.S.M., Purusothaman, K., Tulloch, R., Bolton, W.V. & Wylie, B.R. (1992) Risk factors for hepatitis C virus infection in blood donors: a case–control study. *Med. J. Aust.*, **157**, 227–230

Kanai, K., Kako, M. & Okamoto, H. (1992) HCV genotypes in chronic hepatitis C and response to interferon (Letter to the Editor). *Lancet*, **339**, 1543

Kato, N., Hijikata, M., Ootsuyama, Y., Nakagawa, M., Ohkoshi, S., Sugimura, T. & Shimotohno, K. (1990) Molecular cloning of the human hepatitis C virus genome from Japanese patients with non-A, non-B hepatitis. *Proc. natl Acad. Sci. USA*, **87**, 9524–9528

Kelen, G.D., Green, G.B., Purcell, R.H., Chan, D.W., Qaqish, B.F., Sivertson, K.T. & Quinn, T.C. (1992) Hepatitis B and hepatitis C in emergency department patients. *New Engl. J. Med.*, **326**, 1399–1404

Kew, M.C., Houghton, M., Choo, Q.L. & Kuo, G. (1990) Hepatitis C virus antibodies in southern African blacks with hepatocellular carcinoma. *Lancet*, **335**, 873–874

Kiyosawa, K. & Furuta, S. (1992) Clinical aspects and epidemiology of hepatitis B and C viruses in hepatocellular carcinoma in Japan. *Cancer Chemother. Pharmacol.*, **31** (Suppl. 1), S150–S156

Kiyosawa, K., Sodeyama, T., Tanaka, E., Gibo, Y., Yoshizawa, K., Nakano, Y., Furuta, S., Akahane, Y., Nishioka, K., Purcell, R.H. & Alter, H.J. (1990a) Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: analysis by detection of antibody to hepatitis C virus. *Hepatology*, **12**, 671–675

Kiyosawa, K., Tanaka, E., Sodeyama, T., Furuta, K., Usuda, S., Yousuf, M. & Furuta, S. (1990b) Transition of antibody to hepatitis C virus from chronic hepatitis to hepatocellular carcinoma. *Jpn. J. Cancer Res.*, **81**, 1089–1091

Kiyosawa, K., Sodeyama, T., Tanaka, E., Nakano, Y., Furuta, S., Nishioka, K., Purcell, R.H. & Alter, H.J. (1991) Hepatitis C in hospital employees with needlestick injuries. *Ann. intern. Med.*, **115**, 367–369

Klein, R.S., Freeman, K., Taylor, P.E. & Stevens, C.E. (1991) Occupational risk for hepatitis C virus infection among New York City dentists. *Lancet*, **338**, 1539–1542

Koonin, E.V. (1991) The phylogeny of RNA-dependent RNA polymerases of positive-strand RNA viruses. *J. gen. Virol.*, **72**, 2197–2206

Kotwal, G.J., Baroudy, B.M., Kuramoto, I.K., McDonald, F.F., Schiff, G.M., Holland, P.V. & Zeldis, J.B. (1992a) Detection of acute hepatitis C virus infection by ELISA using a synthetic peptide comprising a structural epitope. *Proc. natl Acad. Sci. USA*, **89**, 4486–4489

Kotwal, G.J., Rustgi, V.K. & Baroudy, B.M. (1992b) Detection of hepatitis C virus-specific antigens in semen from non-A, non-B hepatitis patients. *Dig. Dis. Sci.*, **37**, 641–644

Krawczynski, K., Beach, M.J., Bradley, D.W., Kuo, G., Di Bisceglie, A.M., Houghton, M., Reyes, G.R., Kim, J.P., Choo, Q.-L. & Alter, M.J. (1992) Hepatitis C virus antigen in hepatocytes: immunomorphologic detection and identification. *Gastroenterology*, **103**, 622–629

Kremsdorf, D., Porchon, C., Kim, J.P., Reyes, G.R. & Bréchot, C. (1991) Partial nucleotide sequence analysis of a French hepatitis C virus: implications for HCV genetic variability in the E2/NS1 protein. *J. gen. Virol.*, **72**, 2557–2561

Kubo, Y., Takeuchi, K., Boonmar, S., Katayama, T., Choo, Q.-L., Kuo, G., Weiner, A.J., Bradley, D.W., Houghton, M., Saito, I. & Miyamura, T. (1989) A cDNA fragment of hepatitis C virus isolated from an implicated donor of post-transfusion non-A, non-B hepatitis in Japan. *Nucleic Acids Res.*, **17**, 10367–10372

Kumar, U., Cheng, D., Thomas, H. & Monjardino, J. (1992) Cloning and sequencing of the structural region and expression of putative core gene of hepatitis C virus from a British case of chronic sporadic hepatitis. *J. gen. Virol.*, **73**, 1521–1525

Kuo, G., Choo, Q.-L., Alter, H.J., Gitnick, G.L., Redeker, A.G., Purcell, R.H., Miyamura, T., Dienstag, J.L., Alter, M.J., Stevens, C.E., Tegtmeier, G.E., Bonino, F., Colombo, M., Lee, W.-S., Kuo, C., Berger, K., Shuster, J.R., Overby, L.R., Bradley, D.W. & Houghton, M. (1989) An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. *Science*, **244**, 362–364

Kuroki, T., Nishiguchi, S., Fukuda, K., Shiomi, S., Monna, T., Murata, R., Isshiki, G., Hayashi, N., Shikata, T. & Kobayashi, K. (1991) Mother-to-child transmission of hepatitis C virus (Letter to the Editor). *J. infect. Dis.*, **164**, 427–428

Lamas, E., Baccarini, P., Housset, C., Kremsdorf, D. & Bréchot, C. (1992) Detection of hepatitis C virus (HCV) RNA sequences in liver tissue by in situ hybridization. *J. Hepatol.*, **16**, 219–223

Laurian, Y., Blanc, A., Delaney, S.R. & Allain, J.-P. (1992) All exposed hemophiliacs have markers of HCV (Letter to the Editor). *Vox sang.*, **62**, 55–56

Lee, S.-D., Lee, F.-Y., Wu, J.-C., Hwang, S.-J., Wang, S.-S. & Lo, K.-J. (1992) The prevalence of anti-hepatitis C virus among Chinese patients with hepatocellular carcinoma. *Cancer*, **69**, 342–345

Lefkowitch, J.H. & Apfelbaum, T.F. (1989) Non-A, non-B hepatitis: characterization of liver biopsy pathology. *J. clin. Gastroenterol.*, **11**, 225–232

Lefkowitch, J.H., Schiff, E.R., Davis, G.L., Perrillo, R.P., Lindsay, K., Bodenheimer, H.C., Jr, Balart, L.A., Ortego, T.J., Payne, J., Dienstag, J.L., Gibas, A., Jacobson, I.M., Tamburro, C.H., Carey, W., O'Brien, C., Sampliner, R., Van Thiel, D.H., Feit, D., Albrecht, J., Meschievitz, C., Sanghvi, B., Vaughan, R.D. & the Hepatitis Interventional Therapy Group (1993) Pathological diagnosis of chronic hepatitis C: a multicenter comparative study with chronic hepatitis B. *Gastroenterology*, **104**, 595–603

Leslie, D.E., Rann, S., Nicholson, S., Fairley, C.K. & Gust, I.D. (1992) Prevalence of hepatitis C antibodies in patients with clotting disorders in Victoria. Relationship with other blood borne viruses and liver disease. *Med. J. Aust.*, **156**, 789–792

Leung, N.W.Y., Tam, J.S., Lai, J.Y., Leung, T.W.T., Lau, W.Y., Shiu, W. & Li, A.K.C. (1992) Does hepatitis C virus infection contribute to hepatocellular carcinoma in Hong Kong? *Cancer*, **70**, 40–44

Levrero, M., Tagger, A., Balsano, C., De Marzio, E., Avantaggiati, M.L., Natoli, G., Diop, D., Villa, E., Diodati, G. & Alberti, A. (1991) Antibodies to hepatitis C virus in patients with hepatocellular carcinoma. *J. Hepatol.*, **12**, 60–63

Li, J.-S., Tong, S.-P., Vitvitski, L., Lepot, D. & Trépo, C. (1991) Evidence of two major genotypes of hepatitis C virus in France and close relatedness of the predominant one with the prototype virus. *J. Hepatol.*, **13** (Suppl. 4), S33–S37

Liang, T.J., Jeffers, L., Reddy, R.K., Silva, M.O., Cheinquer, H., Findor, A., De Medina, M., Yarbough, P.O., Reyes, G.R. & Schiff, E.R. (1993) Fulminant or subfulminant non-A, non-B viral hepatitis: the role of hepatitis C and E viruses. *Gastroenterology*, **104**, 556–562

Lin, H.-H., Hsu, H.-Y., Chang, M.-H., Hong, K.-F., Young, Y.-C., Lee, T.-Y., Chen, P.-J. & Chen, D.-S. (1991) Low prevalence of hepatitis C virus and infrequent perinatal or spouse infections in pregnant women in Taiwan. *J. med. Virol.*, **35**, 237–240

Lin, C.K., Chu, R., Li, K.B. & Leong, S. (1992) A study of hepatitis C virus antibodies and serum alanine amino transferase in blood donors in Hong Kong Chinese. *Vox sang.*, **62**, 98–101

Linke, H.K., Miller, M.F., Peterson, D.A., Muchmore, E., Lesniewski, R.R., Carrick, R.J., Gagne, G.D. & Popper, H. (1987) Documentation of non-A, non-B hepatitis in a chimpanzee with hepatocellular carcinoma. In: Robinson, W., Koike, K. & Well, H., eds, *Hepadna Viruses*, New York, Alan R. Liss, pp. 357–370

Liou, T.-C., Chang, T.-T., Young, K.-C., Lin, X.-Z., Lin, C.-Y. & Wu, H.-L. (1992) Detection of HCV RNA in saliva, urine, seminal fluid, and ascites. *J. med. Virol.*, **37**, 197–202

Liu, K., Hu, Z., Li, H., Prince, A.M. & Inchauspé, G. (1992) Genomic typing of hepatitis C viruses in China. *Gene*, **114**, 245–250

Maddrey, W.C. & Van Thiel, D.H. (1988) Liver transplantation. An overview. *Hepatology*, **8**, 948–959

Maéno, M., Kaminaka, K., Sugimoto, H., Esumi, M., Hayashi, N., Komatsu, K., Abe, K., Sekiguchi, S., Yano, M., Mizuno, K. & Shikata, T. (1990) A cDNA clone closely associated with non-A, non-B hepatitis. *Nucleic Acids Res.*, **18**, 2685–2689

Maggi, P. & Petrarulo, F. (1992) Is transfusion the only risk factor for HCV infection among hemodialyzed patients? (Letter to the Editor). *Nephron*, **61**, 240

Martell, M., Esteban, J.I., Quer, J., Genescà, J., Weiner, A., Esteban, R., Guardia, J. & Gómez, J. (1992) Hepatitis C virus (HCV) circulates as a population of different but closely related genomes: quasispecies nature of HCV genome distribution. *J. Virol.*, **66**, 3225–3229

Martin, P., Di Bisceglie, A.M., Kassianides, C., Lisker-Melman, M. & Hoofnagle, J.H. (1989) Rapidly progressive non-A, non-B hepatitis in patients with human immunodeficiency virus infection. *Gastroenterology*, **97**, 1559–1561

Matsuura, Y., Harada, S., Suzuki, R., Watanabe, Y., Inoue, Y., Saito, I. & Miyamura, T. (1992) Expression of processed envelope protein of hepatitis C virus in mammalian and insect cells. *J. Virol.*, **66**, 1425–1431

Mattsson, L., Grillner, L. & Weiland, O. (1992) Seroconversion to hepatitis C virus antibodies in patients with acute post-transfusion non-A, non-B hepatitis in Sweden with a second-generation test. *Scand. J. infect. Dis.*, **24**, 15–20

McFarlane, I.G., Smith, H.M., Johnson, P.J., Bray, G.P., Vergani, D. & Williams, R. (1990) Hepatitis C virus antibodies in chronic active hepatitis: pathogenetic factor or false-positive result? *Lancet*, **335**, 754–757

McHutchison, J.G., Person, J.L., Govindarajan, S., Valinluck, B., Gore, T., Lee, S.R., Nelles, M., Polito, A., Chien, D., DiNello, R., Quan, S., Kuo, G. & Redeker, A.G. (1992) Improved detection of hepatitis C virus antibodies in high-risk populations. *Hepatology*, **15**, 19–25

Mencarini, P., De Luca, A., Antinori, A., Maiuro, G., Spedini, G., Bailly, C. & Tamburrini, E. (1991) Prevalence of anti-HCV antibodies in Cameroon (Short report). *Trans. R. Soc. trop. Med. Hyg.*, **85**, 654–655

Mendenhall, C.L., Seeff, L., Diehl, A.M., Ghosn, S.J., French, S.W., Gartside, P.S., Rouster, S.D., Buskell-Bales, Z., Grossman, C.J., Roselle, G.A., Weesner, R.E., Garcia-Pont, P., Goldberg, S.J., Kiernan, T.W., Tamburro, C.H., Zetterman, R., Chedid, A., Chen, T., Rabin, L. & the VA Cooperative Study Group (1991) Antibodies to hepatitis B virus and hepatitis C virus in alcoholic hepatitis and cirrhosis: their prevalence and clinical relevance. *Hepatology*, **14**, 581–589

Menéndez, S.R., Garciá, M.R., Sánchez San Román, S., Menéndez Tévar, F., Suárez Gonsález, A., Alvarez Navascués, C., Pérez Alvarez, R. & Rodrigo Sáez, L. (1991) Intrafamilial spread of hepatitis C virus. *Infection*, **19**, 431–433

Miller, R.H. & Purcell, R.H. (1990) Hepatitis C virus shares amino acid sequence similarity with pestiviruses and flaviviruses as well as members of two plant virus supergroups. *Proc. natl Acad. Sci. USA*, **87**, 2057–2061

Mishiro, S., Hoshi, Y., Takeda, K., Yoshikawa, A., Gotanda, T., Takahashi, K., Akahane, Y., Yoshizawa, H., Okamoto, H., Tsuda, F., Peterson, D.A. & Muchmore, E. (1990) Non-A, non-B hepatitis specific antibodies directed at host-derived epitope: implication for an autoimmune process. *Lancet*, **336**, 1400–1403

Mita, E., Hayashi, N., Ueda, K., Kasahara, A., Fusamoto, H., Takamizawa, A., Matsubara, K., Okayama, H. & Kamada, T. (1992) Expression of MBP-HCV NS1/E2 fusion protein in *E. coli* and detection of anti-NS1/E2 antibody in type C chronic liver disease. *Biochem. biophys. Res. Commun.*, **183**, 925–930

Mondelli, M.U. & Colombo, M. (1991) The emerging picture of hepatitis C. *Dig. Dis.*, **9**, 245-252

Mondello, P., Patti, S., Vitale, M.G., D'Accardo, A.M. & Spanó, C. (1992) Anti-HCV antibodies in household contacts of patients with cirrhosis of the liver—preliminary results. *Infection*, **20**, 51–52

Mori, S., Kato, N., Yagyu, A., Tanaka, T., Ikeda, Y., Petchclai, B., Chiewsilp, P., Kurimura, T. & Shimotohno, K. (1992) A new type of hepatitis C virus in patients in Thailand. *Biochem. biophys. Res. Commun.*, **183**, 334–342

Mosconi, G., Campieri, C., Miniero, R., Coli, L., Orsi, C., La Manna, G., De Sanctis, L.B., Stefoni, S., Sprovieri, G. & Bonomini, V. (1992) Epidemiology of hepatitis C in a population of hemodialysis patients. *Nephron*, **61**, 298–299

Muchmore, E., Popper, H., Peterson, D.A., Miller, M.F. & Lieberman, H.M. (1988) Non-A, non-B hepatitis-related hepatocellular carcinoma in a chimpanzee. *J. med. Primatol.*, **17**, 235–246

Murakawa, K., Esumi, M., Kato, T., Kambara, H. & Shikata, T. (1992) Heterogeneity within the nonstructural protein 5-encoding region of hepatitis C viruses from a single patient. *Gene*, **117**, 229–232

Nalpas, B., Driss, F., Pol, S., Hamelin, B., Housset, C., Bréchot, C. & Berthelot, P. (1991) Association between HCV and HBV infection in hepatocellular carcinoma and alcoholic liver disease. *J. Hepatol.*, **12**, 70-74

Negro, F., Pacchioni, D., Shimizu, Y., Miller, R.H., Bussolati, G., Purcell, R.H. & Bonino, F. (1992) Detection of intrahepatic replication of hepatitis C virus RNA by in situ hybridization and comparison with histopathology. *Proc. natl Acad. Sci. USA*, **89**, 2247–2251

Ng, Y.-Y., Lee, S.-D., Wu, S.-C., Yang, W.-C., Chiang, S.-S. & Huang, T.-P. (1991) Antibodies to hepatitis C virus in uremic patients on continuous ambulatory peritoneal dialysis. *J. med. Virol.*, **35**, 263–266

Nishioka, K., Watanabe, J., Furuta, S., Tanaka, E., Iino, S., Suzuki, H., Tsuji, T., Yano, M., Kuo, G., Choo, Q-L., Houghton, M. & Oda, T. (1991) A high prevalence of antibody to the hepatitis C virus in patients with hepatocellular carcinoma in Japan. *Cancer*, **67**, 429–433

Novati, R., Thiers, V., d'Arminio Monforte, A., Maisonneuve, P., Principi, N., Conti, M., Lazzarin, A. & Bréchot, C. (1992) Mother-to-child transmission of hepatitis C virus detected by nested polymerase chain reaction. *J. infect. Dis.*, **165**, 720–723

Ohkoshi, S., Kojima, H., Tawaraya, H., Miyajima, T., Kamimura, T., Asakura, H., Satoh, A., Hirose, S., Hijikata, M., Kato, N. & Shimotohno, K. (1990) Prevalence of antibody against non-A, non-B hepatitis virus in Japanese patients with hepatocellular carcinoma. *Jpn. J. Cancer Res.*, **81**, 550–553

Okamoto, H. (1992) Characterization of genomic structure of hepatitis C virus. *Igaku no Ayumi*, **161**, 298–302 (in Japanese)

Okamoto, H., Okada, S., Sugiyama, Y., Yotsumoto, S., Tanaka, T., Yoshizawa, H., Tsuda, F., Miyakawa, Y. & Mayumi, M. (1990a) The 5′-terminal sequence of the hepatitis C virus genome. *Jpn. J. exp. Med.*, **60**, 167–177

Okamoto, H., Munekata, E., Tsuda, F., Takahashi, K., Yotsumoto, S., Tanaka, T., Tachibana, K., Akahane, Y., Sugai, Y., Miyakawa, Y. & Mayumi, M. (1990b) Enzyme-linked immunosorbent assay for antibodies against the capsid protein of hepatitis C virus with a synthetic oligopeptide. *Jpn. J. exp. Med.*, **60**, 223–233

Okamoto, H., Okada, S., Sugiyama, Y., Tanaka, T., Sugai, Y., Akahane, Y., Machida, A., Mishiro, S., Yoshizawa, H., Miyakawa, Y. & Mayumi, M. (1990c) Detection of hepatitis C virus RNA by a two-stage polymerase chain reaction with two pairs of primers deduced from the 5′-noncoding region. *Jpn. J. exp. Med.*, **60**, 215–222

Okamoto, H., Okada, S., Sugiyama, Y., Kurai, K., Iizuka, H., Machida, A., Miyakawa, Y. & Mayumi, M. (1991) Nucleotide sequence of the genomic RNA of hepatitis C virus isolated from a human carrier: comparison with reported isolates for conserved and divergent regions. *J. gen. Virol.*, **72**, 2697–2704

Okamoto, H., Kurai, k., Okada, S.-I., Yamamoto, K., Lizuka, H., Tanaka, T., Fukuda, S., Tsuda, F. & Mishiro, S. (1992a) Full-length sequence of a hepatitis C virus genome having poor homology to reported isolates: comparative study of four distinct genotypes. *Virology*, **188**, 331–341

Okamoto, H., Kojima, M., Okada, S.-I., Yoshizawa, H., Iizuka, H., Tanaka, T., Muchmore, E.E., Peterson, D.A., Ito, Y. & Mishiro, S. (1992b) Genetic drift of hepatitis C virus during a 8.2-year infection in a chimpanzee: variability and stability. *Virology*, **190**, 894–899

Okamoto, H., Tsuda, F., Machida, A., Munekata, E., Akahane, Y., Sugai, Y., Mashiko, K., Mitsui, T., Tanaka, T., Miyakawa, Y. & Mayumi, M. (1992c) Antibodies against synthetic oligopeptides deduced from the putative core gene for the diagnosis of hepatitis C virus infection. *Hepatology*, **15**, 180–186

Omata, M., Yokosuka, O., Takano, S., Kato, N., Hosoda, K., Imazeki, F., Tada, M., Ito, Y. & Ohto, M. (1991) Resolution of acute hepatitis C after therapy with natural beta interferon (Short report). *Lancet*, **338**, 914-915

Parkin, D.M., Srivatanakul, P., Khlat, M., Chenvidhya, D., Chotiwan, P., Insiripong, S., L'Abbé, K.A. & Wild, C.P. (1991) Liver cancer in Thailand. I. A case–control study of cholangiocarcinoma. *Int. J. Cancer*, **48**, 323–328

Perez Alvarez, L., Gurbindo, M.D., Hernandez-Sampelayo, T., Casado, C., Leon, P., Garcia Saiz, A., De Andres, R. & Najera, R. (1992) Mother-to-infant transmission of HIV and hepatitis C infections in children born to HIV-seropositive mothers. *AIDS*, **6**, 427–440

Perrillo, R.P., Pohl, D.A., Roodman, S.T. & Tsai, C.C. (1981) Acute non-A, non-B hepatitis with serum sickness-like syndrome and aplastic anemia. *J. Am. med. Assoc.*, **245**, 494–496

van der Poel, C.L., Bresters, D., Reesink, H.W., Plaisier, A.A.D., Schaasberg, W., Leentvaar-Kuypers, A., Choo, Q.-L., Quan, S., Polito, A., Houghton, M., Kuo, G., Lelie, P.N. & Cuypers, H.T.M. (1992) Early anti-hepatitis C virus response with second-generation C200/C22 ELISA. *Vox sang.*, **62**, 208–212

Pohjanpelto, P. (1992) Risk factors connected with hepatitis C infections in Finland (Short communication). *Scand. J. infect. Dis.*, **24**, 251–252

Pol, S., Legendre, C., Saltiel, C., Carnot, F., Bréchot, C., Berthelot, P., Mattlinger, B. & Kreis, M. (1992) Hepatitis C virus in kidney recipients. Epidemiology and impact on renal transplantation. *J. Hepatol.*, **15**, 202-206

Ponz, E., Campistol, J.M., Barrera, J.M., Gil, C., Pinto, J., Andreu, J. & Bruguera, M. (1991) Hepatitis C virus antibodies in patients on hemodialysis and after kidney transplantation. *Transplant. Proc.*, **23**, 1371–1372

Poynard, T., Aubert, A., Lazizi, Y., Bedossa, P., Hamelin, B., Terris, B., Naveau, S., Dubreuil, P., Pillot, J. & Chaput, J.-C. (1991) Independent risk factors for hepatocellular carcinoma in French drinkers. *Hepatology*, **13**, 896–901

Prince, A.M., Brotman, B., Grady, G.F., Kulins, W.J., Hazzi, C., Levine, R.W. & Millian, S.J. (1974) Long-incubation post-transfusion hepatitis without serological evidence of exposure to hepatitis B virus. *Lancet*, **ii**, 241–246

Prince, A.M., Brotman, B., Huima, T., Pascual, D., Jaffery, M. & Inchauspé, G. (1992) Immunity to hepatitis C infection. *J. infect. Dis.*, **165**, 438–443

Qian, C., Camps, J., Maluenda, M.D., Civeira, M.P. & Prieto, J. (1992) Replication of hepatitis C virus in peripheral blood mononuclear cells. Effect of alpha-interferon therapy. *J. Hepatol.*, **16**, 380–383

Ranger, S., Aussel, L., Winbreck, P., Loustaud, V., Rogues, A.M., Mounier, M., Delpeyroux, C. & Denis, F. (1991) Seroprevalence of hepatitis C virus in subjects contaminated with the human immunodeficiency virus. *Pathol. biol.*, **39**, 126–130 (in French)

Rapicetta, M., Attili, A.F., Mele, A., De Santis, A., Chionne, P., Cristiano, K., Spada, E., Giuliani, E., Carli, L., Goffredo, F. & Capocaccia, L. (1992) Prevalence of hepatitis C virus antibodies and hepatitis C virus-RNA in an urban population. *J. med. Virol.*, **37**, 87–92

Read, A.E., Donegan, E., Lake, J., Ferrell, L., Galbraith, C., Kuramoto, I.K., Zeldis, J.B., Ascher, N.L., Roberts, J. & Wright, T.L. (1991) Hepatitis C in patients undergoing liver transplantation. *Ann. intern. Med.*, **114**, 282–284

Reesink, H.W., Wong, V.C.W, Ip, H.M.H., van der Poel, C.L., van Exel-Oehlers, P.J. & Lelie, P.N. (1990) Mother-to-infant transmission and hepatitis C virus (Letter to the Editor). *Lancet*, **335**, 1216–1217

Robson, S.C., Du Toit, J.M.G., Brice, E.A.W., Bird, A.R. & Brink, N.S. (1991) Hepatitis C virus antibodies in patients with liver disease. The western Cape experience. *South Afr. med. J.*, **80**, 282–284

Roudot-Thoraval, F., Deforges, L., Girollet, J.-P., Maria, B., Milliez, J., Pathier, D., Duval, J. & Dhumeaux, D. (1992) Prevalence of antibodies to hepatitis C virus in a population of pregnant women in France. *Gastroenterol. clin. Biol.*, **16**, 255–259 (in French)

Ruiz-Moreno, M., Rua, M.J., Castillo, I., García-Novo, M.D., Santos, M., Navas, S. & Carreño, V. (1992) Treatment of children with chronic hepatitis C with recombinant interferon-alpha: a pilot study. *Hepatology*, **16**, 882–885

Saito, I., Miyamura, T., Ohbayashi, A., Harada, H., Katayama, T., Kikuchi, S., Watanabe, Y., Koi, S., Onji, M., Ohta, Y., Choo, Q.-L., Houghton, M. & Kuo, G. (1990) Hepatitis C virus infection is associated with the development of hepatocellular carcinoma. *Proc. natl Acad. Sci. USA*, **87**, 6547–6549

Sbolli, G., Zanetti, A.R, Tanzi, E., Cavanna, L., Civardi, G., Fornari, F., Di Stasi, M. & Buscarini, L. (1990) Serum antibodies to hepatitis C virus in Italian patients with hepatocellular carcinoma. *J. med. Virol.*, **30**, 230–232

Schlipköter, U., Gladziwa, U., Cholmakov, K., Weise, A., Rasshofer, R., Lorbeer, B., Luz, N., Deinhardt, F. & Raggendorf, M. (1992) Prevalence of hepatitis C virus infection in dialysis patients and their contacts using a second generation enzyme-linked immunosorbent assay. *Med. Microbiol. Immunol.*, **181**, 173–180

Schoub, B.D., Johnson, S., McAnerney, J.M. & Blackburn, N.K. (1992) The role of sexual transmission in the epidemiology of hepatitis C virus in black South Africans. *Trans. R. Soc. trop. Med. Hyg.*, **86**, 431–433

Scott, D.A., Constantine, N.T., Callahan, J., Burans, J.P., Olson, J.G., Al-Fadeel, M., Al-Ozieb, H., Arunkumer, H. & Hyams, K.C. (1992) The epidemiology of hepatitis C virus antibody in Yemen. *Am. J. trop. Med. Hyg.*, **46**, 63–68

Seeff, L.B., Buskell-Bales, Z., Wright, E.C., Durako, S.J., Alter, H.J., Iber, F.L., Hollinger, F.B., Gitnick, G., Knodell, R.G., Perrillo, R.P., Stevens, C.E., Hollingsworth, C.G. & the National Heart, Lung and Blood Institute Study Group (1992) Long-term mortality after transfusion-associated non-A, non-B hepatitis. *New Engl. J. Med.*, **327**, 1906–1911

Sheu, J.-C., Lee, S.-H., Wang, J.-T., Shih, L.-N., Wang, T.-H. & Chen, D.-S. (1992a) Prevalence of anti-HCV and HCV viremia in hemodialysis patients in Taiwan. *J. med. Virol.*, **37**, 108–112

Sheu, J.-C., Huang, G.-T., Shih, L.-N., Lee, W.-C., Chou, H.-C., Wang, J.-T., Lee, P.-H., Lai, M.-Y., Wang, C.-Y., Yang, P.-M., Lee, H.-S. & Chen, D.-S. (1992b) Hepatitis C and B viruses in hepatitis B surface antigen-negative hepatocellular carcinoma. *Gastroenterology*, **103**, 1322–1327

Shieh, Y.S.C., Shim, K.-S., Lampertico, P., Balart, L.A., Jeffers, L.J., Thung, S.N., Regenstein, F., Reddy, K.R., Farr, G., Schiff, E.R. & Gerber, M.A. (1991) Detection of hepatitis C virus sequences in liver tissue by the polymerase chain reaction. *Lab. Invest.*, **65**, 408–411

Shimizu, Y.K., Weiner, A.J., Rosenblatt, J., Wong, D.C., Shapiro, M., Popkin, T., Houghton, M., Alter, H.J. & Purcell, R.H. (1990) Early events in hepatitis C virus infection in chimpanzees. *Proc. natl Acad. Sci. USA*, **87**, 6441–6444

Shimizu, S., Kiyosawa, K., Sodeyama, T., Tanaka, E. & Nakano, M. (1992) High prevalence of antibody to hepatitis C virus in heavy drinkers with chronic liver diseases in Japan. *J. Gastroenterol. Hepatol.*, **7**, 30–35

Shimizu, Y.K., Iwamoto, A., Hijikata, M., Purcell, R.H. & Yoshikura, H. (1992) Evidence for in vitro replication of hepatitis C virus genome in a human T-cell line. *Proc. natl Acad. Sci. USA*, **89**, 5477–5481

Shindo, M., Di Bisceglie, A.M. & Hoofnagle, J.H. (1992a) Long-term follow-up of patients with chronic hepatitis C treated with alpha-interferon. *Hepatology*, **15**, 1013–1016

Shindo, M., Di Bisceglie, A.M., Biswas, R., Mihalik, K. & Feinstone, S.M. (1992b) Hepatitis C virus replication during acute infection in the chimpanzee. *J. infect. Dis.*, **166**, 424–427

Simonetti, R.G., Cottone, M., Craxi, A., Pagliaro, L., Rapicetta, M., Chionne, P. & Costantino, A. (1989) Prevalence of antibodies to hepatitis C virus in hepatocellular carcinoma (Letter to the Editor). *Lancet*, **ii**, 1338

Simonetti, R.G., Cammà, C., Fiorello, F., Cottone, M., Rapicetta, M., Marino, L., Fiorentino, G., Craxì, A., Ciccaglione, A., Giuseppetti, R., Stroffolini, T. & Pagliaro, L. (1992) Hepatitis C virus infection as a risk factor for hepatocellular carcinoma in patients with cirrhosis. A case-control study. *Ann. intern. Med.*, **116**, 97–102

Spaete, R.R., Alexander, D'A., Rugroden, M.E., Choo, Q.-L., Berger, K., Crawford, K., Kuo, C., Leng, S., Lee, C., Ralston, R., Thudium, K., Tung, J.W., Kuo, G. & Houghton, M. (1992) Characterization of the hepatitis C virus E2/NS1 gene product expressed in mammalian cells. *Virology*, **188**, 819–830

Srivatanakul, P., Parkin, D.M., Khlat, M., Chenvidhya, D., Chotiwan, P., Insiripong, S., L'Abbé, K.A. & Wild, C.P. (1991) Liver cancer in Thailand. II. A case-control study of hepatocellular carcinoma. *Int. J. Cancer*, **48**, 329–332

Stroffolini, T., Chiaramonte, M., Tiribelli, C., Villa, E., Simonetti, R.G., Rapicetta, M., Stazi, M.A., Bertin, T., Croce, S.L., Trande, P., Magliocco, A. & Chionne, P. (1992) Hepatitis C virus infection, HBsAg carrier state and hepatocellular carcinoma: relative risk and population attributable risk from a case-control study in Italy. *J. Hepatol.*, **16**, 360–363

Sun, Z.T., Zhu, Y.R. & Harris, C.C. (1993) Viral chemical interaction in hepatocarcinogenesis in area of prevalence (Abstract 331/33). *Cancer Detect. Prev.*, **17**, 207

Suzuki, H. *et al.* (1991) Clinical significance of second generation anti-HCV assay kit (ELISA) and RIBA-II in liver disease. *J. Med. pharm. Sci.*, **26**, 303–312 (in Japanese)

Tabor, E., Gerety, R.J., Drucker, J.A., Seeff, L.B., Hoofnagle, J.H., Jackson, D.R., April, M., Barker, L.F. & Pineda-Tamondong, G. (1978) Transmission of non-A, non-B hepatitis from man to chimpanzee. *Lancet*, **i**, 463–466

Takada, N., Takase, S., Enomoto, N., Takada, A. & Date, T. (1992) Clinical backgrounds of the patients having different types of hepatitis C virus genomes. *J. Hepatol.*, **14**, 35–40

Takahashi, K., Okamoto, H., Kishimoto, S., Munekata, E., Tachibana, K., Akahane, Y., Yoshizawa, H. & Mishiro, S. (1992) Demonstration of a hepatitis C virus-specific antigen predicted from the putative core gene in the circulation of infected hosts. *J. gen. Virol.*, **73**, 667–672

Takamizawa, A., Mori, C., Fuke, I., Manabe, S., Murakami, S., Fujita J., Onishi, E., Andoh, T., Yoshida, I. & Okayama, H. (1991) Structure and organization of the hepatitis C virus genome isolated from human carriers. *J. Virol.*, **65**, 1105–1113

Takeda, S., Nagafuchi, Y., Tashiro, H., Abe, Y., Fukushige, H., Komori, H., Okamoto, K., Ohsato, K. & Haratake, J. (1992) Antihepatitis C virus status in hepatocellular carcinoma and the influence on clinicopathological findings and operative results. *Br. J. Surg.*, **79**, 1195–1198

Takehara, T., Hayashi, N., Mita, E., Hagiwara, H., Ueda, K., Katayama, K., Kasahara, A., Fusamoto, H. & Kamada, T. (1992) Detection of the minus strand of hepatitis C virus RNA by reverse transcription and polymerase chain reaction: implications for hepatitis C virus replication in infected tissue. *Hepatology*, **15**, 387–390

Takeuchi, K., Boonmar, S., Kubo, Y., Katayama, T., Harada, H., Ohbayashi, A., Choo, Q.-L., Kuo, G., Houghton, M., Saito, I. & Miyamura, T. (1990a) Hepatitis C viral cDNA clones isolated from a healthy carrier donor implicated to post-transfusion non-A, non-B hepatitis. *Gene*, **91**, 287–291

Takeuchi, K., Kubo, Y., Boonmar, S., Watanabe, Y., Katayama, T., Choo, Q.-L., Kuo, G., Houghton, M., Saito, I. & Miyamura, T. (1990b) Nucleotide sequence of core and envelope genes of the hepatitis C virus genome derived directly from human healthy carriers. *Nucleic Acids Res.*, **18**, 4626

Tamura, I., Koda, T., Kobayashi, Y., Ichimura, H., Kurimura, O. & Kurimura, T. (1992) Prevalence of four blood-borne viruses (HBV, HCV, HTLV-1, HIV-1) among haemodialysis patients in Japan. *J. med. Virol.*, **36**, 271–273

Tanaka, K., Hirohata, T., Koga, S., Sugimachi, K., Kanematsu, T., Ohryohji, F., Nawata, H., Ishibashi, H., Maeda, Y., Kiyokawa, H., Tokunaga, K., Irita, Y., Takeshita, S., Arase, Y. & Nishino, N. (1991) Hepatitis C and hepatitis B in the etiology of hepatocellular carcinoma in the Japanese population. *Cancer Res.*, **51**, 2842–2847

Tanaka, E., Kiyosawa, K., Sodeyama, T., Hayata, T., Ohike, V., Nakano, Y., Yoshizawa, K., Furuta, S., Watanabe, Y., Watanabe, J. & Nishioka, K. (1992) Prevalence of antibody to hepatitis C virus in Japanese schoolchildren: a comparison with adult blood donors. *Am. J. trop. Med. Hyg.*, **46**, 460–464

Tanaka, T., Kato, N., Nakagawa, M., Ootsuyama, Y., Cho, M.-J., Nakazawa, T., Hijikata, M., Ishimura, Y. & Shimotohno, K. (1992) Molecular cloning of hepatitis C virus genome from a single Japanese carrier: sequence variation within the same individual and among infected individuals. *Virus Res.*, **23**, 39–53

Tateda, A., Kikuchi, K., Numazaki, Y., Shirachi, R. & Ichida, N. (1979) Non-B hepatitis in Japanese recipients of blood transfusions: clinical and serologic studies after the introduction of laboratory screening of donor blood for hepatitis B surface antigen. *J. infect. Dis.*, **139**, 511–518

Terada, S., Kawanishi, K. & Katayama, K. (1992) Minimal hepatitis C infectivity in semen (Letter to the Editor). *Ann. intern. Med.*, **117**, 171–172

Thaler, M.M., Park, C.-K., Landers, D.V., Wara, D.W., Houghton, M., Veeremann-Wauters, G., Sweet, R.-L. & Han, J.H. (1991) Vertical transmission of hepatitis C (Short report). *Lancet*, **338**, 17–18

Theilmann, L., Solbach, C., Toex, U., Müller, H.M., Pfaff, E., Otto, G. & Goeser, T. (1992) Role of hepatitis C virus infection in German patients with fulminant and subacute hepatic failure. *Eur. J. clin. Invest.*, **22**, 569–571

Trichopoulos, D., Day, N.E., Kaklamani, E., Tzonou, A., Muñoz, N., Zavitsanos, X., Koumantaki, Y. & Trichopoulou, A. (1987) Hepatitis B virus, tobacco smoking and ethanol consumption in the etiology of hepatocellular carcinoma. *Int. J. Cancer*, **39**, 45–49

Tsukiyama-Kohara, K., Kohara, M., Yamaguchi, K., Maki, N., Toyoshima, A., Miki, K., Tanaka, S., Hattori, N. & Nomoto, A. (1991) A second group of hepatitis C viruses. *Virus Genes*, **5**, 243–254

Tzonou, A., Trichopoulos, D., Kaklamani, E., Zavitsanos, X., Koumantaki, Y. & Hsieh, C.-C. (1991) Epidemiologic assessment of interactions of hepatitis-C virus with seromarkers of hepatitis-B and -D viruses, cirrhosis and tobacco smoking in hepatocellular carcinoma. *Int. J. Cancer*, **49**, 377–380

Ueda, T., Ohta, K., Suzuki, N., Yamaguchi, M., Hirai, K., Horiuchi, T., Watanabe, J., Miyamoto, T. & Ito, K. (1992) Idiopathic pulmonary fibrosis and high prevalence of serum antibodies to hepatitis C virus. *Am. Rev. respir. Dis.*, **146**, 266–268

Vandelli, L., Medici, G., Savazzi, A.M., De Palma, M., Vecchi, C., Zanchetta, G. & Lusvarghi, E. (1992) Behavior of antibody profile against hepatitis C virus in patients on maintenance hemodialysis. *Nephron*, **61**, 260–262

Vargas, V., Castells, L. & Esteban, J.I. (1990) High frequency of antibodies to the hepatitis C virus among patients with hepatocellular carcinoma (Letter to the Editor). *Ann. intern. Med.*, **112**, 232–233

Verbaan, H., Widell, A., Lindgren, S., Lindmark, B., Nordenfelt, E. & Eriksson, S. (1992) Hepatitis C in chronic liver disease: an epidemiological study based on 566 consecutive patients undergoing liver biopsy during a 10-year period. *J. intern. Med.*, **232**, 33–42

Viladomiu, L., Genescà, J., Esteban, J.I., Allende, H., González, A., López-Talavera, J.C., Esteban, R. & Guardia, J. (1992) Interferon-alpha in acute post-transfusion hepatitis C: a randomized, controlled trial. *Hepatology*, **15**, 767–769

Wang, J.-T., Sheu, J.-C., Lin, J.-T., Wang, T.-H. & Chen, D.S. (1992) Detection of replicative form of hepatitis C virus RNA in peripheral blood mononuclear cells. *J. infect. Dis.*, **166**, 1167–1169

Wang, Y., Okamoto, H. & Mishiro, S. (1992) HCV genotypes in China (Letter to the Editor). *Lancet*, **339**, 1168

Watanabe, J., Minegishi, K., Mitsumori, T., Ishifuji, M., Oguchi, T., Ueda, M., Tokunaga, E., Tanaka, E., Kiyosawa, K., Furuta, S., Katayama, T., Kuo, G., Choo, Q.-L., Houghton, M. & Nishioka, K. (1990) Prevalence of anti-HCV antibody in blood donors in the Tokyo area. *Vox sang.*, **59**, 86–88

Watanabe, J. *et al.* (1991a) Sensitivity and specificity of assays for antibody to HCV by 2nd generation ELISA and RIBA-II in voluntary blood donors. *J. Med. pharm. Sci.*, **26**, 313–321 (in Japanese)

Watanabe, J., Matsumoto, C., Nishioka, K. & Yoshizawa, H. (1991b) Anti-GOR to screen HCV-RNA-positive blood donors (Letter to the Editor). *Lancet*, **338**, 391

Watanabe, Y., Harada, S., Saito, I. & Miyamura, T. (1991) Prevalence of antibody against the core protein of hepatitis C virus in patients with hepatocellular carcinoma. *Int. J. Cancer*, **48**, 340–343

Watanabe, J., Matsumoto, C., Fujimura, K., Shimada, T., Yoshizawa, H., Okamoto, H., Iizuka, H., Tango, T., Ikeda, H., Endo, N., Mazda, T., Nojiri, T., Aoyama, K., Kanemitsu, K., Yamano, H., Mizui, M., Yokoishi, F., Tokunaga, K. & Nishioka, K. (1993) Predictive value of screening tests for persistent hepatitis C virus infection evidenced by viraemia: Japanese experience. *Vox sang.*, **65**, 199–203

Watson, H.G., Ludlam, C.A., Rebus, S., Zhang, L.Q., Peutherer, J.F. & Simmonds, P. (1992) Use of several second generation serological assays to determine the true prevalence of hepatitis C virus infection in haemophiliacs treated with non-virus inactivated factor VIII and IX concentrates. *Br. J. Haematol.*, **80**, 514–518

Weiner, A.J., Christopherson, C., Hall, J.E., Bonino, F., Saracco, G., Brunetto, M.R., Crawford, K., Marion, C.D., Crawford, K.A., Venkatakrishna, S., Miyamura, T., McHutchinson, J., Cuypers, T. & Houghton, M. (1991) Sequence variation in hepatitis C viral isolates. *J. Hepatol.*, **13** (Suppl. 4), S6–S14

Weiner, A.J., Geysen, H.M., Christopherson, C., Hall, J.E., Mason, T.J., Saracco, G., Bonino, F., Crawford, K., Marion, C.D., Crawford, K.A., Brunetto, M., Barr, P.J., Miyamura, T., McHutchinson, J. & Houghton, M. (1992) Evidence for immune selection of hepatitis C virus (HCV) putative envelope glycoprotein variants: putative role in chronic HCV infections. *Proc. natl Acad. Sci. USA*, **89**, 3468–3472

Weintrub, P.S., Veereman-Wauters, G., Cowan, M.J. & Thaler, M.M. (1991) Hepatitis C virus infection in infants whose mothers took street drugs intravenously. *J. Pediatr.*, **119**, 869–874

Widell, A., Hansson, B.G., Berntorp, E., Moestrup, T., Johansson, H.P., Hansson, H. & Nordenfelt, E. (1991) Antibody to a hepatitis C virus related protein among patients at high risk for hepatitis B. *Scand. J. infect. Dis.*, **23**, 19–24

Wright, T.L., Hsu, H., Donegan, E., Feinstone, S., Greenberg, H., Read, A., Ascher, N.L., Roberts, J.P. & Lake, J.R. (1991) Hepatitis C virus not found in fulminant non-A, non-B hepatitis. *Ann. intern. Med.*, **115**, 111–112

Wright, H.I., Gavaler, J.S. & Van Thiel, D.H. (1992) Preliminary experience with interferon-alpha-2b therapy of viral hepatitis in liver allograft recipients. *Transplantation*, **53**, 121–124

Wright, T.L., Hollander, H., Kim, M., Wilber, J., Chan, C., Polito, A., Urdea, M., Thaler, M.M., Held, M.J. & Scharschmidt, B. (1992a) Hepatitis C viremia in human immunodeficiency virus (HIV) infected patients with and without AIDS (Abstract No. 101). *Hepatology*, **16**, 70A

Wright, T.L., Donegan, E., Hsu, H.H., Ferrell, L., Lake, J.R., Kim, M., Combs, C., Fennessy, S., Roberts, J.P., Ascher, N.L. & Greenberg, H.B. (1992b) Recurrent and acquired hepatitis C viral infection in liver transplant recipients. *Gastroenterology*, **103**, 317–322

Wyke, R.J., Tsiquaye, K.N., Thornton, A., White, Y., Portmann, B., Das, P.K., Zuckerman, A.J. & Williams, R. (1979) Transmission of non-A non-B hepatitis to chimpanzees by factor-IX concentrates after fatal complications in patients with chronic liver disease. *Lancet*, **i**, 520–524

Xu, Z., Sheng, F.-M., Xu, Z.-Y. & Wang, Q.-S. (1990) Relationship between HCV infection and primary hepatocellular carcinoma (PHC). *Tumor (Shanghai)*, **10**, 115 (in Chinese)

Yoshioka, K., Kakumu, S., Wakita, T., Ishikawa, T., Itoh, Y., Takayanagi, M., Higashi, Y., Shibata, M. & Morishima, T. (1992) Detection of hepatitis C virus by polymerase chain reaction and response to interferon-alpha therapy: relationship to genotypes of hepatitis C virus. *Hepatology*, **16**, 293–299

Yoshizawa, H., Akahane, Y., Itoh, Y., Iwakiri, S., Kitajima, K., Morita, M., Tanaka, A., Nojiri, T., Shimizu, M., Miyakawa, M. & Mayumi, M. (1980) Virus-like particles in a plasma fraction (fibrinogen) and in the circulation of apparently healthy blood donors capable of inducing non-A/non-B hepatitis in humans and chimpanzees. *Gastroenterology*, **79**, 512–520

Yousuf, M., Nakano, Y., Tanaka, E., Sodeyama, T. & Kiyosawa, K. (1992) Persistence of viremia in patients with type-C chronic hepatitis during long-term follow-up. *Scand. J. Gastroenterol.*, **27**, 812–816

Yu, M.-W. & Chen, C.-J. (1993) Elevated serum testorenone levels and risk of hepatocellular carcinoma. *Cancer Res.*, **53**, 790–794

Yu, M.C., Tong, M.J., Coursaget, P., Ross, R.K., Govindarajan, S. & Henderson, B.E. (1990) Prevalence of hepatitis B and C viral markers in black and white patients with hepatocellular carcinoma in the United States. *J. natl Cancer Inst.*, **82**, 1038–1041

Yu, M.-W., You, S.-L., Chang, A.-S., Lu, S.-N., Liaw, Y.-F. & Chen, C.-J. (1991) Association between hepatitis C virus antibodies and hepatocellular carcinoma in Taiwan. *Cancer Res.*, **51**, 5621–5625

Yuki, N., Hayashi, N., Kasahara, A., Hagiwara, H., Katayama, K., Fusamoto, H. & Kamada, T. (1992) Hepatitis B virus markers and antibodies to hepatitis C virus in Japanese patients with hepatocellular carcinoma. *Dig. Dis. Sci.*, **37**, 65–72

Zaaijer, H.L., Cuypers, H.T.M., Reesink, H.W., Winkel, I.N., Gerken, G. & Lelie, P.N. (1993) Reliability of polymerase chain reaction for detection of hepatitis C virus (Short report). *Lancet*, **341**, 722–724

Zavitsanos, X., Hatzakis, A., Kaklamani, E., Tzonou, A., Toupadaki, N., Broeksma, C., Chrispeels, J., Troonen, H., Hadziyannis, S., Hsieh, C.-C., Alter, H. & Trichopoulos, D. (1992) Association between hepatitis C virus and hepatocellular carcinoma using assays based on structural and nonstructural hepatitis C virus peptides. *Cancer Res.*, **52**, 5364–5367

Zhang, Y.-Y., Hansson, B.G., Widell, A. & Nordenfelt, E. (1992) Hepatitis C virus antibodies and hepatitis C virus RNA in Chinese blood donors determined by ELISA, recombinant immunoblot assay and polymerase chain reaction. *Acta pathol. microbiol. immunol. scand.*, **100**, 851–855

Zignego, A.L., Macchia, D., Monti, M., Thiers, V., Mazzetti, M., Foschi, M., Maggi, E., Romagnani, S., Gentilini, P. & Bréchot, C. (1992) Infection of peripheral mononuclear blood cells by hepatitis C virus. *J. Hepatol.*, **15**, 382–386