Autonomic nervous system function in patients with chronic hepatitis C virus infection

Ph.D. dissertation

János Osztovits M.D.

Gastroenterology Program,
Clinical School of Ph.D. Studies
Semmelweis University, Budapest, Hungary

Tutor: Prof. Ferenc Szalay, M.D., Ph.D., Sc.D.

Examination board: Prof. János Fehér†, MD, Ph.D., Sc.D.
Margit Abonyi, MD, Ph.D.
Endre Ibrányi, MD, Ph.D.

Official reviewers: Gabriella Lengyel MD, Ph.D.
Gabriella Pár MD, Ph.D.

Budapest, 2011

Appendix

Abbreviations

ALT: alanine aminotransferase

AST: aspartate aminotransferase

BMI: body mass index

BRS: baroreflex sensitivity

BRS_{seq}: baroreflex sensitivity sequence index

CDC: Centers for Disease Control and Prevention, United States

DBP: diastolic blood pressure

ELISA: enzyme-linked immunosorbent assay;

HAV: hepatitis A virus

HBV: hepatitis B virus

HCC: hepatocellular carcinoma

HCV: hepatitis C virus

HF: high-frequency power of R-R interval variability

HIV: human immunodeficiency virus

HRV: heart rate variability

IFN: interferon

LF: low-frequency power of RR-interval variability

LF_{gain}: cross-spectral transfer gain in the low-frequency range, a baroreflex index

NNSD: standard deviation of R-R intervals

NTR: nontranslated region

PCR: polymerase chain reaction

pNN50: percentage of RR intervals that differ >50 ms

RIBA: recombinant immunoblot assay

RMSSD: root mean square of successive R-R interval differences

RNA: ribonucleic acid

RRI: R-R intervals

SBP: systolic blood pressure

Summary

Chronic hepatitis C virus (HCV) infection can be associated with various extrahepatic manifestations, including peripheral and central nervous system complications. Autonomic function has not been investigated in patients with chronic HCV infection before, although impaired autonomic function has already been described in patients with chronic liver diseases from different etiologies, and has proven to be a poor prognostic indicator. In addition, the current treatment of interferon-alpha plus ribavirin is known to affect neurological manifestations in patients with chronic HCV infection.

In our first, cross-sectional study we aimed to determine cardiovagal autonomic function in patients with chronic HCV infection, comparing to healthy controls. In our second study we followed-up the patients during the course of antiviral therapy and aimed to examine the possible changes of cardiovagal autonomic function.

Autonomic function was assessed in 45 treatment-naive patients with chronic HCV infection and in 40 healthy controls by determining spontaneous baroreflex sensitivity (BRS) and heart rate variability (HRV) indices with non-invasive methods. Then, we followed up 22 patients and assessed BRS and HRV indices at the beginning of treatment and at week 12, 24 and 48 of antiviral therapy. Besides, laboratory analyses and quantitative polymerase chain reaction for serum HCV RNA level were performed.

BRS and HRV indices were lower in patients with HCV infection compared to healthy controls, and independently correlated with serum ALT levels. Further, both HRV and BRS indices decreased after 12 weeks of therapy compared to pretreatment values; then they increased significantly by week 24 and continued to improve by week 48 of therapy. These changes were independent from the presence of cryoglobulins and from the virological response.

Our results suggest that impaired autonomic function is caused by chronic HCV infection. The increase of autonomic dysfunction at the beginning of the antiviral therapy may be caused by the immunomodulatory actions of interferon alfa-2. Further studies are needed, however, to understand the exact mechanisms.

Összefoglalás

A krónikus hepatitis C vírus (HCV) fertőzés a betegek egy részében extrahepatikus eltéréseket okozhat, ezen belül ismertek a perifériás és a központi idegrendszert érintő szövődmények is. Az autonóm idegrendszer működését hepatitis C vírus fertőzött betegekben még nem vizsgálták, habár annak károsodása ismert különböző krónikus májbetegségekben, és igazolták azt is, az autonóm károsodás rossz prognosztikai tényező. A HCV fertőzés elleni kombinált interferon-alfa és ribavirin kezelés szintén hatással van az idegrendszeri szövődményekre.

Ezért az első, keresztmetszeti vizsgálatunkban meghatároztuk a kardiovagális autonóm idegrendszer működését krónikus HCV fertőzött betegekben, egészséges kontroll személyekhez képest. Majd második tanulmányunkban követtük a betegeket az antivirális kezelés során, és vizsgáltuk az autonóm idegrendszer működésének változásait.

A keresztmetszeti vizsgálat során 45 krónikus HCV fertőzött beteg és 40 egészséges kontroll személy szívfrekvencia variabilitását (HRV) és baroreflex érzékenységét (BRS) határoztuk meg, non-invazív módszerekkel. Ezt követően 22 beteget követtünk az antivirális kezelés során, és meghatároztuk a BRS és HRV értékeket a kezelés kezdetekor, valamint a kezelés 12., 24., és 48. hetében. Emellett laboratóriumi vizsgálatokat és mennyiségi HCV RNS meghatározást végeztünk.

Mind a BRS, mind a HRV értékek alacsonyabbak voltak a HCV fertőzött betegekben az egészséges kontrollokhoz képest, és csak a szérum GPT értékkel mutattak független összefüggést. A követéses vizsgálatban a kezelés 12. hetében csökkent HRV és BRS értékeket mértünk a kiindulási adatokhoz képest, majd szignifikáns emelkedést a 24. héten, és további emelkedő tendenciát a 48. héten. A változások függetlenek voltak a cryoglobulin jelenlététől és a vírusválasztól.

Vizsgálatunk csökkent autonóm idegrendszeri funkciót igazolt krónikus hepatitis C vírus fertőzött betegekben. Az antivirális kezelés kezdetén észlelt további autonóm funkció csökkenés hátterében az interferon alfa-2 immunmoduláló hatása állhat. További vizsgálatok szükségesek annak tisztázására, mi okozhatja az autonóm funkció észlelt eltéréseit krónikus HCV fertőzött betegekben.

1. Introduction

Hepatitis C virus (HCV) is one of the leading causes of chronic viral infection worldwide. According to estimations from the World Health Organization (WHO), approximately 3% of the world population has been infected with HCV, amounting to 170 million people globally (1). About 3 to 4 million people are infected each year and it is estimated that between 65% and 80% of people newly infected with HCV progress to chronic hepatitis, a leading cause of end-stage liver disease and hepatocellular carcinoma (HCC). On the other hand, about 27% of cases of cirrhosis and 25% of instances of hepatocellular carcinoma arise in HCV-infected people (2,3). Consequently, HCV infection contributes significantly to worldwide morbidity.

1.1. Discovering hepatitis C virus - 20 years of history

In year 1989, researchers (leaded by Michael Houghton) first isolated a single cDNA clone that was shown to be derived from a new flavi-like virus, termed the hepatitis C virus. The discovery of hepatitis C was the direct result of the landmark discoveries of hepatitis B virus (HBV) and hepatitis A virus (HAV) and their serologies. Screening tests for HAV and HBV made it possible in the mid-1970s to examine cases of transfusion-associated hepatitis and to demonstrate that only approximately 25% resulted from HBV and that none were related to HAV. Consequently, approximately 75% of transfusion-associated hepatitis became classified as non-A, non-B hepatitis (NANBH) (4). Subsequently, chimpanzee studies demonstrated that NANBH was a result of a transmissible agent. It gradually became apparent that the NANBH agent often resulted in chronic hepatitis and sometimes evolved into cirrhosis.

The NANBH agent remained a virologic enigma for the next decade, until researchers leaded by Houghton at the Chiron Corporation (California, USA) used an ambitious molecular approach on large volumes of high-titer infectious chimpanzee plasma from the Centers for Disease Control and Prevention (USA). They extracted RNA, cloned it into an expression vector, and screened the expressed product with presumed immune sera (5). A single positive clone was found in the millions screened,

and, by year 1990, the entire genome was sequenced and the agent was identified as a novel flavivirus - the hepatitis C virus (HCV).

Retrospective analysis of pedigreed samples at the National Institute of Health (NIH) showed that 70% to 90% of NANBH cases were HCV related. The impact of HCV blood donor screening has been enormous. The single-antigen first-generation enzyme immunoassay (EIA-1) prevented 40,000 HCV infections within the first year, and the second-generation assay (EIA-2) has actually reduced new transfusion-related HCV infections to almost zero (6,7,8).

1.2. Transmission and epidemiology

HCV is transmitted most efficiently by percutaneous exposure to blood, such as blood transfusion, transplantation of infected organs, and injecting drug use. Transmission is much less efficient by mucosal exposure to blood or serum-derived fluids (eg, a child born to an infected mother or sex with an infected partner) (9,10).

Blood transfusion was a major risk factor for HCV transmission before the introduction of sensitive and specific anti-HCV testing in June, 1992 (11). This route of transmission has been virtually eliminated in countries where screening of blood donors is implemented, thus, transfusion-related hepatitis has almost disappeared, leaving injection drug use as the most common mode of transmission.

Injecting drug use is currently the primary risk factor for HCV in the USA and Australia and accounts for most new diagnoses of HCV infection in Europe (12). It has been reported, that 48% of the patients with acute HCV infection in the USA in 2007 were users of injected drugs (13). Global rates of infection in injection drug users are high, with rates of at least 50% reported in 86% of countries in which prevalence studies have been carried out (14). In addition to needle sharing, sharing of other drug paraphernalia—such as foil and spoons—has been implicated in HCV transmission (15).

In developing and transitional economy countries, the nosocomial transmission of new HCV infections is a major problem because of the re-use of contaminated or inadequately sterilized syringes and needles used in medical, paramedical and dental procedures, with an estimated 2.3–4.7 million of new infections occurring each year (16). The highest reported rates of needle re-use are found in the Middle East, South-

East Asia and the Western Pacific. In Egypt, the treatment of endemic schistosomiasis in mass programs (discontinued in the 1980s) that frequently used unsterilized needles and syringes has led to a national HCV prevalence of more than 14%, with rates of 20–30% in young male adults (17).

Sexual and perinatal HCV transmission does happen but significantly less extent than with hepatitis B. The rate of sexual transmission is controversial, and in stable heterosexual relationships it is thought to be low, with 1–3% of partners of HCV-infected people reported to have the infection in cross-sectional studies. Co-infection with HIV, duration of relationship, or chronic liver disease could be independent cofactors increasing risk for transmission (18).

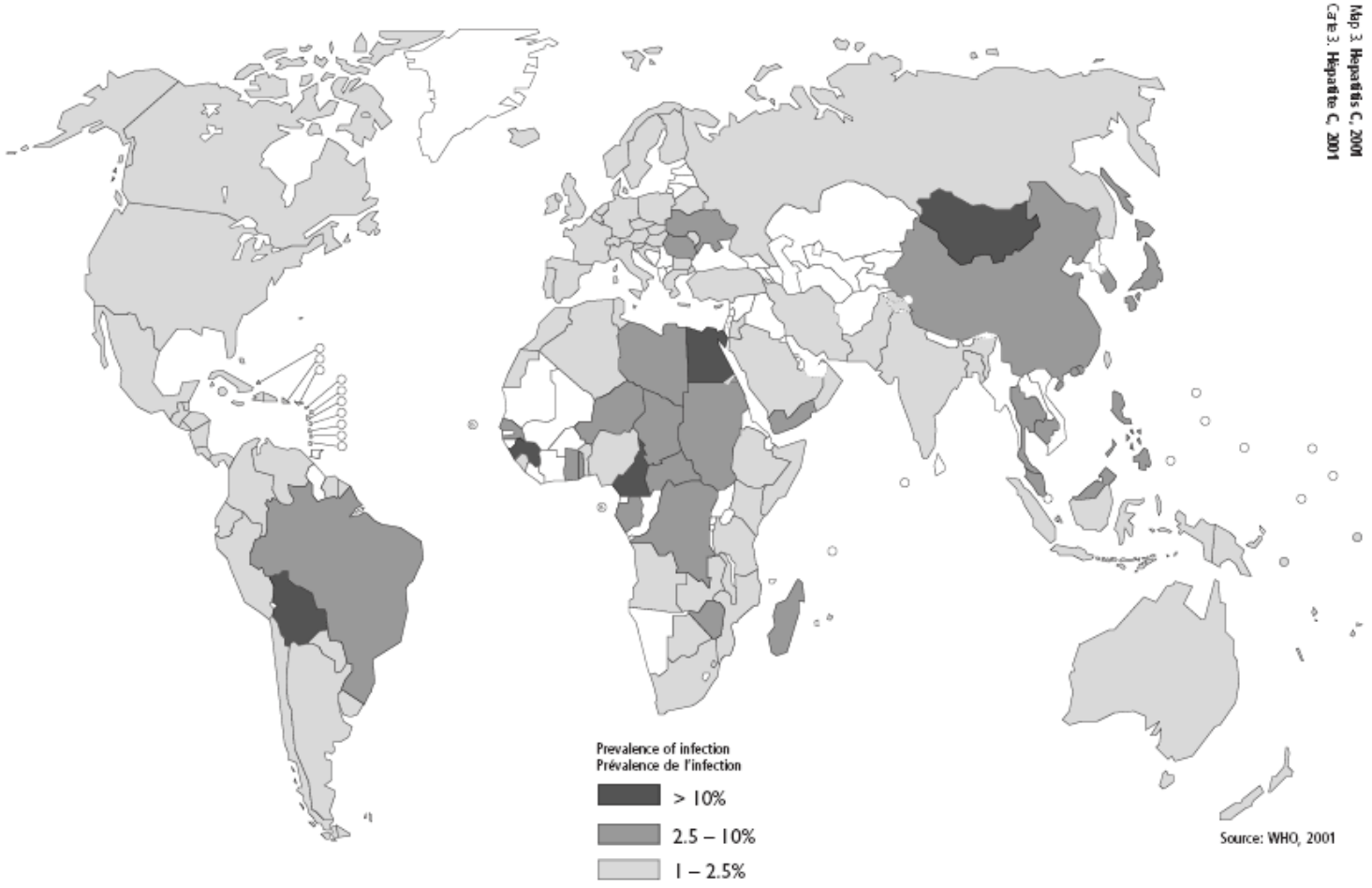
Perinatal transmission takes place at a rate of about 4% and is two to four-folds higher in mothers co-infected with HIV (19). Currently, no evidence is available from randomized controlled trials to suggest that caesarean section offers an advantage over vaginal delivery (20). The risk posed to the infant from breastfeeding is negligible and non-sexual intrafamilial transmission is very rare (21).

HCV and HIV co-infection is an important issue, with about one in ten individuals infected with HIV also infected with HCV worldwide, whereas in developed countries this figure is one in four and up to 50–95% in certain risk groups (although most HCV-infected individuals are not infected with HIV) (22). HIV infection has an important effect on HCV viral load and on clinical disease progression (23).

Overall, the WHO estimated global prevalence of HCV infection was 3% or 170 million individuals in 1999. The prevalence was higher in some countries in Africa (5.3% or 31.9 million), the eastern Mediterranean (4.6% or 21.3 million), South-East Asia (2.15% or 32.3 million) and the Western Pacific (3.9% or 62.2 million), as compared with some countries in the Americas (1.7% or 13.1 million) and Europe (1.03% or 8.9 million) **(Figure 1.)** (24).

In 2004, the Global Burden of Hepatitis C Working Group, serving as a consultant to the WHO, estimated the global prevalence to be slightly lower at 2.2% or 130 million individuals. The lowest HCV prevalence of 0.01% to 0.1% is from countries in the United Kingdom and Scandinavia, while the highest prevalence of 15% to 20% is from Egypt (25).

Figure 1. Worldwide prevalence of Hepatitis C



1.3. Methods for preventing transmission

No vaccine for HCV is currently available, mainly due to the large number of genotypes and variants of hepatitis C and the fact that HCV itself appears to be only weakly immunogenic. At present, reducing the burden of HCV infection requires the implementation of both primary and secondary prevention, where the first implementation of prevention reduces or eliminates HCV transmission and the second reduces liver and other chronic disease in HCV-infected persons (26).

Specific methods of reducing incident cases of hepatitis C include: (i) screening donated blood and blood products; (ii) encouraging healthcare professionals to take precautions when handling blood and body fluids; (iii) educating people about high-risk behaviors; (iv) educating people about safe sex practices; (v) needle exchange programs and informing drug users on safer injection techniques.

Individuals with HCV infection should take particular caution to help prevent further progression of the disease. For example, they should avoid alcohol or taking new medications (including herbal treatments) without consulting their doctor. In addition, infected patients should be evaluated for the presence or development of chronic liver disease. Some assessments include liver function tests, testing the severity of liver disease, and determining if hepatitis A and B vaccination is necessary. While patients should be aware of their disease progression, they should also be aware of how to prevent passing their disease to others. HCV-infected individuals should not donate blood, body organs, other tissues, or semen; they should not share any personal items that may have their blood on it; and they should cover cuts and sores on their skin.

1.4. Virology of hepatitis C virus

1.4.1. Genetic and molecular structure

HCV is a single stranded RNA virus, approximately 40-60 nm in diameter, consisting of three basic components: an envelope protein complex (E1 and E2 [NS1]), a nucleocapsid core protein (C), and an RNA genome, approximately 9.6 kb in length **(Figure 2.)**

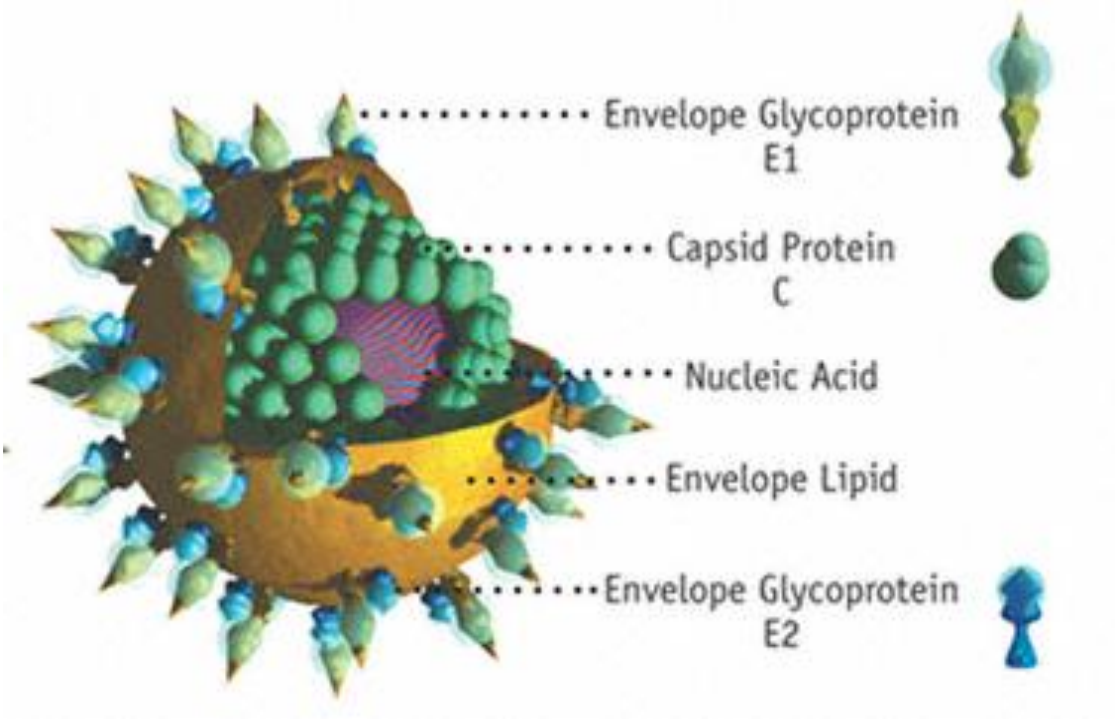


Figure 2. Structure of a hepatitis C virion

Adapted from the Physicians Research Network Notebook, Vol. 6, No.1. March, 2001.

Analysis of the RNA genome revealed that HCV is different form hepatitis A and hepatitis B; HCV is now classified as the only member of the *Hepacivirus* genus, and shares genetic organization and amino acid sequence structure similarity with pestiviruses and flaviviruses, as well as members of two plant virus supergroups: potyviruses and carmoviruses (27).

The 9.6 kb long single positive-stranded genome consists of one large open reading frame, flanked by nontranslated regions (NTRs) at its 5' and 3' ends. The 5' NTR sequence has been shown to be highly-conserved, with an overall nucleotide variation of just 2% (28). In addition to containing the internal ribosomy entry site that mediates the cap-independent translation of the open reading frame, most of the 5' region sequence appears to be necessary for efficient HCV replication (29). The 3' NTR contains a polypyrimidine tract immediately following the open reading frame stop codon, suggesting that conserved RNA elements in the 3' NTR are also essential for HCV replication (30).

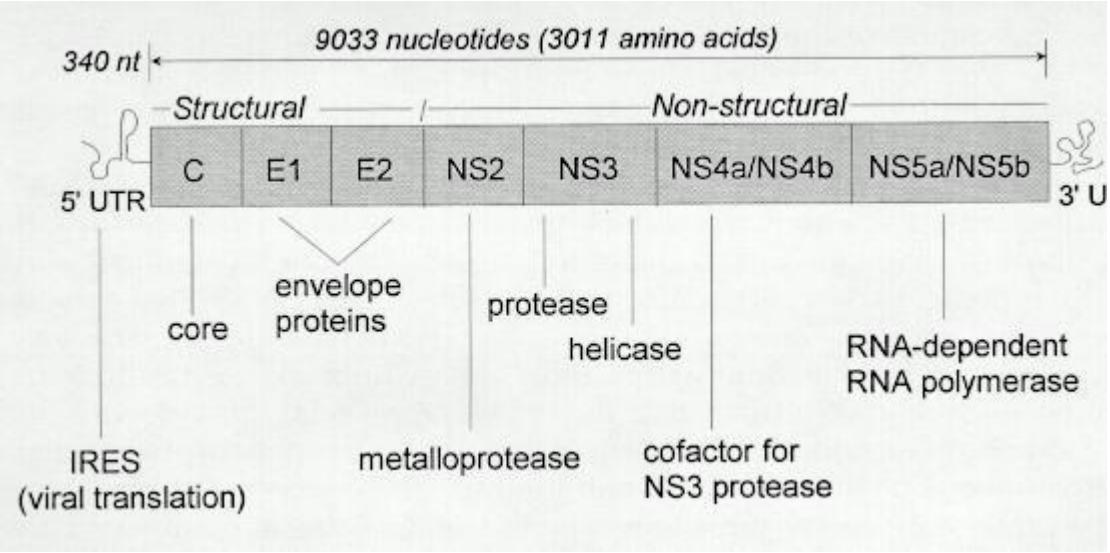


Figure 3. Organization of the hepatitis C virus genome
From Fang et al (31).

The open reading frame a single polyprotein of about 3,010 amino acids in length, which is processed by both host and viral proteases into structural (at least three) and nonstructural (six) proteins. Structural proteins are encoded at the amino (N) terminus, beginning with the small, basic nucleocapsid (core) protein and the envelope glycoproteins E1 and E2; the remainder of the sequence encodes the nonstructural (NS) proteins (**Figure 3.).** The functions of the proteins are summarized in **Table 1**.

1.4.2. Genotype

Examination of the phylogenetic tree of the 5'end of NS4 has confirmed the existence of a substantial genetic diversity. Six major genotypes of HCV are recognized, differing by up to 35% at the primary RNA sequence level. Further, genotypes are branching into at least 50 subtypes. As the viral polymerase enzyme does not have proof-reading capacity, significant number of further variants, quasispecies exists, that differs slightly and emerges under selective pressures of host immunity and drug treatment (32). The major genotypes have been designated the numbers 1 to 6, whereas the subtypes are denoted by letters (**Table 2.).**

Protein	Nucleic acid absolute numbering	Amino acid relative numbering (lenght)	Functions of proteins
(5’ NTR)	1-341	-	-
Core	342–914	191	RNA binding; nucleocapsid; associates with lipid droplets and cellular proteins
E1	915–1490	192	Envelope glycoprotein; associates with E2 to form heterodimer necessary for attachment and cell entry
E2	1491–2579	363	Envelope glycoprotein; receptor binding; associates with E1 to form heterodimer necessary for attachment and cell entry
P7	2580–2768	63	Ion channel; putative viroporin
NS2	2769–3419	217	Component of NS2-3 protease; other functions not determined
NS3	3420–5312	631	N-terminal proteinase domain; C-terminal NTPase/helicase domain; antagonizes host innate antiviral immune response
NS4A	5313–5474	54	NS3-4A proteinase cofactor
NS4B	5475–6257	261	Induces endoplasmic reticulum alterations
NS5A	6258–7601	448	Phosphoprotein; regulation of viral RNA replication; proposed to modulate host cell interferon response and cell signaling
NS5B	7602–9377	591	
(3’ NTR)	9378–9646	-	-

Table 1. Viral proteins and their functions

Adapted from Dubuisson (33) and Kuiken (34)

Terminology	Definition	Nucleotide similarity (%)
Genotype (1-6)	Major genetic group based on similarity of nucleotide sequence	65.7 – 68.9
Subtype (a, b, etc.)	Genetically closely related viruses within of nucleotide sequence	76.9 – 80.1
Quasispecies	Complex of genetic variants within individual isolates	90.8 – 99

Table 2. Classification of hepatitis C virus
From Szabó et al. (2)

Although all six HCV genotypes can be found worldwide, the different genotypes have widely different geographical distributions (Figure 4.). Molecular epidemiology studies have proven great HCV diversity in certain regions of sub-Saharan Africa and in Southeast Asia. Most newly described variants originate from specific geographical regions. For example, infections in western Africa are predominantly genotype 2, whereas those in central Africa, such as the Democratic Republic of Congo and Gabon, are genotypes 1 and 4. Genotypes 3 and 6 show similar genetic diversity in south and eastern Asia. This "endemic" pattern of sequence variability suggests that HCV has probably circulated in human populations in these parts of Africa and Asia for centuries, millennia, or longer (35,36,37).

In contrast, the most common variants found in Western countries (1a and 1b in genotype 1; 2a, 2b, and 2c in genotype 2) have become widely distributed over the past 50–70 years as a result of transmission through blood transfusion and other invasive medical procedures and of needle sharing by injection drug users. They now represent the vast majority of infections encountered clinically in Western countries. Subtypes 1a, 1b, 2a, 2b, 3a, and 4a are likely to be the descendants of HCV variants from endemic areas that "seeded" these new, rapidly expanding transmission networks (38,39). In

Hungary, Gervain et al. demonstrated that over 90% of the Hungarian patients with chronic hepatitis C are infected by HCV 1b subtype (40).

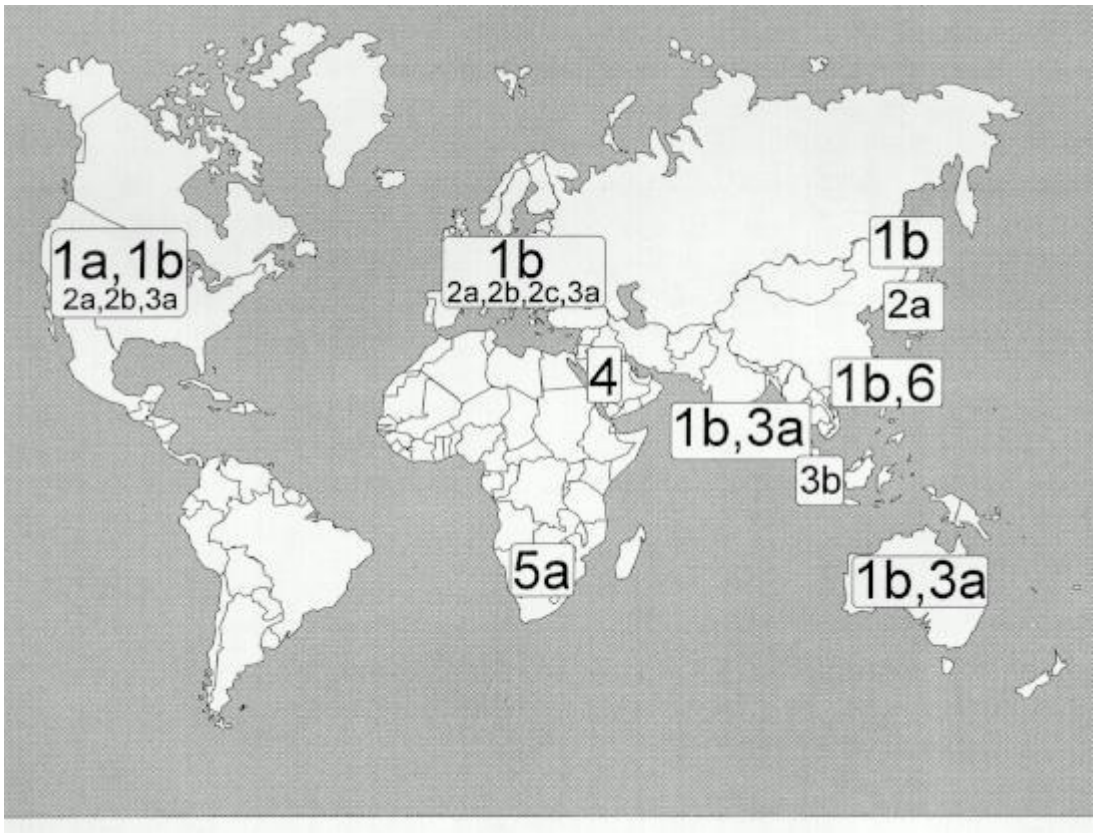


Figure 4. Worldwide distribution of HCV genotypes
From Fang et al (31).

1.5. Natural history

1.5.1. Dynamics of hepatitis C infection

During the first 1-3 weeks following exposure to HCV, some patients can experience increases in serum alanine aminotransferase (ALT) activity; ALT levels for infected patients are usually elevated compared with the uninfected population. Additionally, HCV RNA can be detected in infected patients using reserve transcription-polymerase

chain reaction (RT-PCR). Although levels of both ALT and HCV RNA decrease steadily during the first year after infection, there is no absolute concordance between the two markers: for example, HCV RNA can be detectable in patients with normal ALT levels (41). Furthermore, serum ALT levels fluctuate in both resolved and unresolved cases up to 12 months from the onset of HCV.

Antibodies to HCV (seroconversion) appear 8 or 9 weeks after exposure (42). The rate of positivity for ant-HCV increases in the weeks following infection, with the vast majority of patients showing positivity by week 21 (41). However, rates of positivity for anti-HCV can vary widely depending on the patient and the specific antibody studied. Studies have shown that during the first year after HCV infection, the most commonly detected antibodies are anti-C100 and anticore, originally reported in 94% of patients. This value has now risen to close to 97% of patients since the introduction of second-generation antibody tests. Anti-HCV antibodies may also be detected in a significant proportion of patients whose serum ALT levels return to normal within 12 months of initial infection, although rates of anti-HCV positivity are reportedly higher in patients whose ALT levels remain increased (43).

1.5.2. Acute hepatitis C

Individuals with healthy immune systems who are infected with HCV typically develop an acute infection. Acute infection remains asymptomatic in the majority of patients, although 25-35% of infected individuals may develop mild symptoms. These mild symptoms can include flu-like symptoms, fatigue, nausea, reduced appetite, or abdominal pain. Patients may also become jaundiced.

1.5.3. Chronic hepatitis C, and frequency of spontaneous virological resolution

Early studies found that HCV infection persisted in 75–85% of infected persons over 6 months and became chronic. Conversely, virological resolution occurred in about 15–25%. Similar results were reported in later studies of diverse populations (HCV-infected blood donors, persons with 'community-acquired' infection, IV drug abusers and children with leukemia) (44). Later, a far higher rate of spontaneous resolution was noted among infected children, young women and even some persons with community-

acquired hepatitis C, the figures ranging between 42 and 45% (45). These data suggested that young age at the time of infection is an important determinant of the likelihood of spontaneous recovery.

1.5.4. Fibrosis

Up to 80% of patients with chronic infection go on to develop hepatic fibrosis, ranging from portal tract enlargement to cirrhosis. Rates of fibrosis progression are generally slow, with studies suggesting an average rate between 0.12 and 0.133 per year (46,47). A recent meta-analysis evaluating a total of 111 studies that cumulatively included over 33,000 patients with HCV (48), estimated the annual probability of progressing from each successive fibrosis stage to the next as: Stage F0 (absent) to F1 (mild) fibrosis: 0.117; stage F1 to F2 (moderate) fibrosis: 0.085; Stage F2 to F3 (advanced) fibrosis: 0.120; Stage F3 to F4 (severe fibrosis or cirrhosis): 0.116.

The median time from initial infection to the development of cirrhosis has been estimated as 30 years (47), but is highly variable, depending on the number of risk factors that are thought to accelerate disease progression.

1.5.5. Cirrhosis

Early retrospective studies suggested that between 17% and 55% of patients with chronic HCV go on to develop cirrhosis. However, prospective studies reported much lower progression rates (7-16%), and it is now generally accepted that this value lies more around the 20% mark, with cirrhosis usually occurring approximately 30 years following the initial infection (44) **(Figure 5.)**.

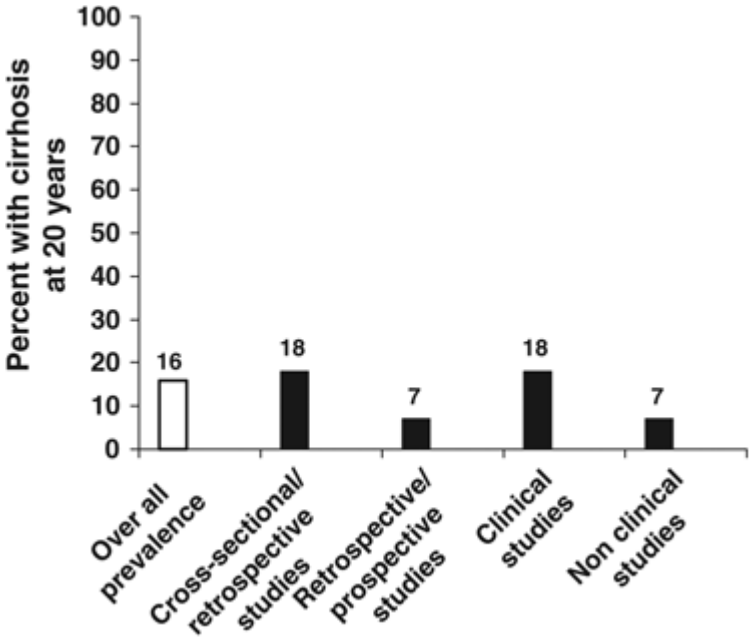


Figure 5. Estimated prevalence of cirrhosis at 20 years after infection according to strategy of case identification. Meta-analysis and metaregression based on 111 reported natural history studies.
From Seeff (45) and Thein (48)

1.5.6. Hepatocellular carcinoma

Despite the fact that only a small proportion of HCV-infected patients go on to develop HCC (usually after 20-40 years of chronic infection), HCV is responsible for a significant proportion of primary liver cancer cases globally and is a leading cause of HCC in Western countries. Thus, HCC represents one of the major HCV complications associated with mortality. The growing morbidity due to HCV infection may be reflected in the rising incidence of HCC and increasing rates of hospitalization for HCC (49). In addition, a rise in the number of liver transplantations performed for HCC and end-stage liver disease may provide further evidence for the increased morbidity associated with HCV infection, as liver failure due to HCV-related cirrhosis is the single most common reason for liver transplants. A retrospective study has shown that the number of transplant recipients with HCV infection increased fivefold between 1990 and 2000 in the USA, and the proportion of recipients with HCV infection increased

from 12% to 37% (50).

The development of HCV-related HCC rarely occurs in the absence of cirrhosis, and an increased risk of HCC has been associated with more advanced stages of cirrhosis **(Figure 6.)**. Estimated annual incidence rates for HCC range between 1% and 4% once cirrhosis is established, although higher rates have been reported depending on additional co-factors, including heavy alcohol consumption, co-infection of hepatitis B virus or HIV, and advanced age (49,51).

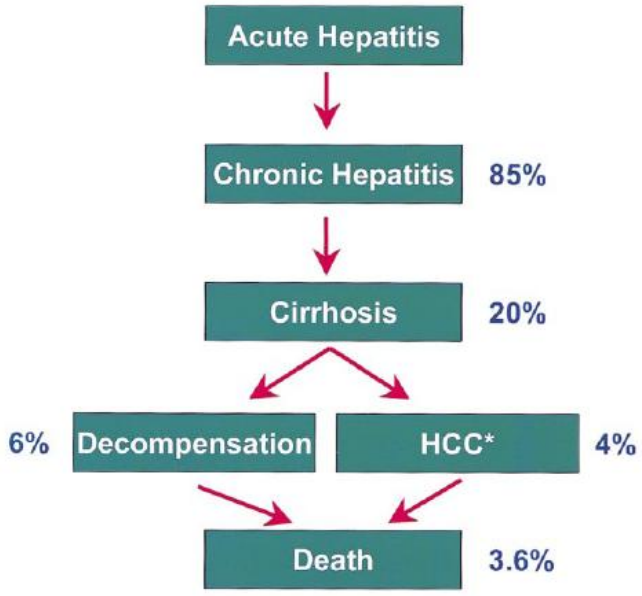


Figure 6. Proportion of an infected population progressing to various complications of hepatitis C viral infection.

From Di Bisceglie (52)

1.5.7. Risk factors for disease progression

Factors that could play a role in progression of chronic hepatitis C include features of the virus, the host or items of environmental or extraneous origin. Regarding the virus, attention has been focused on viral concentration and viral genotype, but there is little definitive evidence that these factors affect liver disease progression.

In contrast, there are numerous host factors that appear to influence fibrosis progression, including age, gender and race. Infection at a young age is associated with a slow rate of progression (47,53). Men with chronic HCV infection are more likely to progress to cirrhosis than are women as is evident from the data of the Irish and German women infected by the contaminated Rh immunoglobulin (54,55). Paradoxically, given their high rate of evolution to HCC and their lower rate of response to treatment, African Americans appear to have a slow rate of progression to cirrhosis (56,57). Genetic polymorphisms have been evaluated for their influence on disease progression, with a focus on major histocompatibilty class I and II alleles (58,59), as well as on profibrogenic cytokines (60,61). Metabolic factors with inter-relationships among steatosis (62), diabetes (63,64) and obesity (65,66) have all been reported as affecting disease progression. Normal values of ALT and its associated lesser degree of histological inflammation appear to predict a lower level and rate of fibrosis progression (67,68). Finally, the rate of progression is increased in the face of co-infection with HIV (69), hepatitis B (70), as well as with the co-morbidity of hemochromatosis (71).

Regarding extraneous issues, the most significant impact on disease progression is the cofactor of associated alcoholism (72), although the exact extent of alcohol intake likely to add to the injury is uncertain (73). Similarly, smoking has been reported to be associated with increased fibrosis progression to both cirrhosis and HCC (74).

1.5.8. HCV-associated mortality

Over an average follow-up of approximately 11 years, a study of 924 blood transfusion recipients infected with HCV and 475 uninfected recipients found that HCV infection carried only a small increased risk in overall mortality compared with the control population (hazard ratio of 1.41) (75). Factors that worsened chances of survival

included male gender, advanced age, and excessive alcohol consumption. HCV patients, however, did show a markedly increased risk of liver-related death compared with controls, at a hazard ratio of 12.84. These findings were supported by a US study of transfusion recipients with confirmed HCV infection, who displayed no significant difference in all-cause mortality versus uninfected controls but significantly rates of liver-related death, 4.1% versus 1.3% (76).

1.6. Immunopathogenesis

1.6.1. HCV cellular entry mechanisms

HCV primarily infects hepatocytes, although viral genomes and antigens are detectable in different lymphocyte cells (monocytes, B lymphocytes) as well as in basal ganglion cells in the central nervous system. Hungarian study group by Lotz, Szalay et al. proved that peripheral red blood cells can also be a reservoir of HCV infection (77).

The precise mechanism by which the virus enters these cells and evades the host's immune responses is not known, but endocytosis of HCV is believed to be dependent on lipoproteins (78). In addition, in the presence of antibodies to LDL receptor, viral cell entry is dose-dependently reduced, and endocytosis of HCV is competitively inhibited by LDL and very-low-density lipoprotein, but not by high-density lipoprotein (79). Endocytosis is also mediated by the formation of HCV-lipoprotein complexes that contain apolipoprotein E (80). It has also been shown that HCV infection causes upregulation of many genes that are related to lipid metabolism. These genes are involved in membrane glycosphingolipid biosynthesis and the induction of lipid metabolism enzymes, lipid transporters, and apolipoproteins (81).

Further studies have demonstrated that the HCV envelope glycoprotein 2 (E2) binds on the cell surface to the CD81, a putative HCV receptor which belongs to the so called tetraspanin family. Additionally, HCV-lipoprotein complexes enable the virus to be transferred by CD81 to tight junction proteins, enabling viral cell entry via endocytosis mediated by the envelope glycoproteins (78).

1.6.2. The HCV life cycle

The HCV life cycle **(Figure 7.)** begins with entry into the host cell, followed by uncoating of the HCV particle's nucleocapsid. It appears likely that the processing of the polyprotein, assembly of the replicase and synthesis of the negative-strand RNA intermediate all occur in association with the endoplasmic reticulum, outside of the nucleus of the host cell (82). The presence of viral, double-stranded RNA in the cytoplasm triggers several innate antiviral mechanisms including activation of PKR, TLR3 and RIG-I, which eventually leads to the release of IFN- alpha and IFN-beta. Translation of the positive-strand viral genome generates nonstructural and structural viral proteins, which are critical for viral replication and assembly of new virus particles, respectively.

1.6.3. Host immune-responses, and mechanism of immune escape

Host immune responses invoked by HCV are complex, consisting a number of components of the innate and adaptive immunity pathways, thus, direct cytopathic effect does not play a major role, HCV infection is rather considered as a systemic immunological disease. Innate immunity is comprised of single physical barriers (e.g., skin and mucus membranes), cellular components (e.g., granulocytes, machrophages and natural killer [NK] cells) and soluble components (e.g., complement factors and type I interferons [IFN-alpa, IFN-beta]). Adaptive immunity is composed of humoral immunity (e.g. antibodies produced by B cells) and, most important in viral infections, cellular immunity (e.g., CD4+, CD8+, and regulatory T cells) (83).

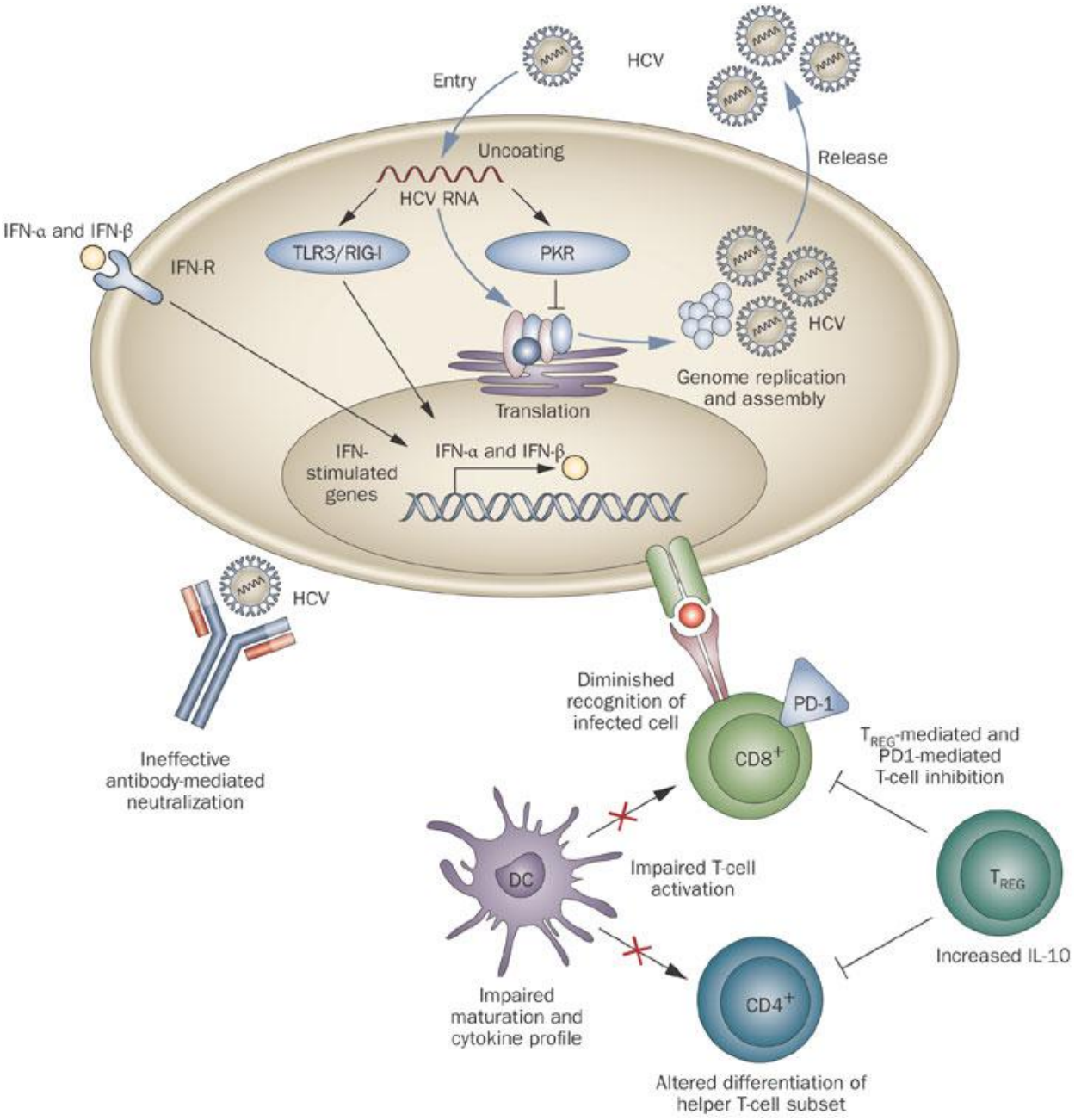


Figure 7. Viral life cycle and major host cell defense pathways

Abbreviations: DC, dendritic cell; IFN, interferon; IL-10, interleukin 10; PKR, double stranded RNA-activated protein kinase; T_{REG}, regulatory T cells.

From Sklan et al. (82)

HCV can modify immune-response in various ways. **Figure 7.** illustrates the key components of the host's immune response and the cellular impairment that is

associated with HCV infection. HCV impairs the function of dendritic cells, and limits their ability to stimulate a robust, antigen-specific immune response in $CD4^+$ and $CD8^+$ T cells. Upregulation of PD-1 and increased IL-10 production further impair T-cell function, which facilitates HCV immune evasion. HCV affects the host immune response through the different viral polypeptides. The HCV NS3/4A serine protease, the HCV-E2 and the NS5A proteins reduce the interferon (IFN) production, induced by viral RNA molecules. The HCV core blocks the signaling way of IFN, moreover, reduces the IL-2 and IFN-gamma production of the antigen-specific T-cells and the IL-12 production of macrophages. The HCV-E2 can reduce the activity, the cytotoxic effect, and the IFN-gamma and TNF-alpha production of NK-cells.

In addition, HCV changes rapidly the antigenic structure of the envelope proteins on the surface of viral particle via hypervariable region in the gene encoding E2 (84). Besides, due to the extreme fast replication (10^{10}-10^{12} virions/day) and the viral polymerase enzyme, that does not have proof-reading capacity, significant numbers of further variants (quasispecies) are produced, and that helps the virus against the humoral and cellular immune-response. This phenomenon is called the hypervariability of HCV. Various quasispecies can co-exist simultaneously within the same patient, and immune selection of a specific variant may allow the generation of subsequent escape mutants.

1.7. Diagnosis of HCV

HCV infection is typically diagnosed when serum ALT levels are persistently increased and anti-HCV antibodies are present in the serum. Diagnosis is then confirmed by the presence of HCV RNA in the serum. However, diagnosis may be difficult, as anti-HCV antibodies are not always present; for example, in some of the patients co-infected with HIV or with other advanced immune deficiency states. Further, patients do not have always elevated ALT levels. In the absence of specific serologic testing, the nonspecific symptoms and ALT elevations associated with HCV infection may be differential diagnostic problem with other hepatic disorders, including chronic hepatitis B and D, alcoholic or drug-induced hepatitis, non-alcoholic steatohepatitis, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, Wilson's disease or hemochromatosis.

1.7.1. Signs and symptoms

1.7.1.1. Clinical manifestations of acute HCV infection

HCV infection has a relatively short incubation period, ranging from 2 to 12 weeks with an average of 6-8 weeks. Symptoms are usually mild, non-specific and intermittent, and most frequently include fatigue, mild right-upper body discomfort or tenderness, nausea, reduced appetite, and muscle and joint pain. Occasionally, nonhepatic symptoms, including low-grade fever, rash and arthralgias may occur, which typically resolve within the icteric phase following the onset of jaundice. Physical examinations are likely to be normal, or may reveal only mild enlargement of the liver or tenderness (85).

1.7.1.2. Clinical manifestations of chronic HCV infection

In approximately 60-85% of patients, HCV infection will develop into a chronic infection. As with acute infection, chronic infection may remain asymptomatic for many years. Non-specific symptoms again include fatigue, poor appetite, and mild right-upper body discomfort. Some patients, however, may present with the complications of decompensated cirrhosis or HCC, presumably after prolonged infection lasting for 20-30 years. Physical signs are associated with either decompensated cirrhosis, or HCC, or the extrahepatic manifestations of chronic HCV infection, which are discussed later on.

1.7.2. Diagnostic tests

1.7.2.1. Virologic testing in hepatitis C

The United States Centers for Disease Control and Prevention (CDC) recommends, that the following patients should be tested for HCV infection (26):

- Persons who have injected illicit drugs in the recent and remote past, including those who injected only once and do not consider themselves to be drug users.

- Persons with conditions associated with a high prevalence of HCV infection including:
    - Persons with HIV infection
    - Persons with hemophilia who received clotting factor concentrates prior to 1987
    - Persons who have ever been on hemodialysis
    - Persons with unexplained abnormal aminotransferase levels
- Prior recipients of transfusions or organ transplants prior to July 1992 including:
    - persons who were notified that they received blood from a donor who later tested positive for HCV infection;
    - persons who received a transfusion of blood or blood products;
    - persons who received an organ transplant
- Healthcare, emergency medical, and public safety workers after needle sticks injury or mucosal exposure to HCV-positive blood
- Children born to HCV-infected mothers
- Current sexual partners of HCV-infected persons (Although the prevalence of infection is low, a negative test in the partner provides reassurance, making testing of sexual partners of benefit in clinical practice.)

Test to confirm HCV infection includes serologic anti-HCV testing, HCV RNA nucleic acid testing, and laboratory evaluations of ALT levels. After acute exposure, HCV RNA is usually detected in serum before antibody; HCV RNA can be identified as early as 2 weeks following exposure whereas anti-HCV is generally not detectable before 8-12 weeks. These two markers of HCV infection may be present in varying permutations, requiring careful analysis for interpretation **(Table 3)**.

Anti-HCV	HCV RNA	Interpretation
Positive	Positive	Acute or chronic HCV depending on the clinical context
Positive	Negative	Resolution of HCV; Acute HCV during period of low-level viraemia
Negative	Positive	Early acute HCV infection; chronic HCV in setting of immunosuppressed state; false positive HCV RNA test
Negative	Negative	Absence of HCV infection

Table 3. Interpretation of HCV Assays
From Ghany et al. (88)

1.7.2.2. Serologic tests

Patients with chronic hepatitis C are initially identified by positive antibody testing. The screening tests are enzyme-linked immunosorbent assays (ELISAs), now in the third generation, with a specificity of over 99%. These assays detect a mixture of antibodies directed against various epitopes on the HCV core or nonstructural proteins. The recombinant immunoblot assay (RIBA), was also developed as a supplemental assay to confirm the results of ELISA. However, with the advent of nucleic acid testing and the improved specificity of ELISA, use of RIBA in HCV diagnosis and management has largely become redundant, although it may still be useful to differentiate between false-positive anti-HCV reactivity and HCV clearance (86).

A difficulty with using anti-HCV assays is that false-positives may occur when testing is performed in populations where the prevalence of HCV is low. Conversely, false-negatives may occur in patients with severe immunosuppression, such those infected with HIV, solid organ transplant recipients, patients with hypo- or agammaglobulinemia, or patients on hemodialysis (86). Consequently, RT-PCR has become a standard for the identification of active HCV infection.

1.7.2.3. Nucleic acid tests

There are two categories of nucleic acid tests: qualitative (qualitative PCR and transcription-mediated amplification) and quantitative (branched-chain DNA amplification, quantitative PCR and real-time PCR). The quantification of HCV RNA using RT-PCR confirms the presence of viraemia and establishes the precise viral load. Although there is no correlation between disease severity and viral load, the levels of viraemia are important in predicting response to therapy. For example, patients with high viral load are more resistant to complete viral clearance with current treatment (87).

Qualitative testing for the presence of HCV RNA is no longer recommended (88). However, genotyping of the virus may be useful, as it has management implications, thus, genotyping is recommended to be performed, prior to INF-based treatment (88).

1.7.2.4. Liver biopsy

Liver biopsy has been widely regarded as the gold standard for determining the severity of liver damage caused by HCV infection (88). Liver biopsies can provide helpful information on the current status of the liver, identify features useful in the treatment decision-making process, and may reveal advanced fibrosis or cirrhosis that warrants screening for HCC and/or varices. In most patients, a biopsy can be safely done via percutaneous route.

However, biopsy is associated with some drawbacks, as it carries a risk of causing pain, bleeding or perforation of other organs. It is also subject to sampling error, requires expertise for interpreting the histopathology, it is costly, and causes anxiety in patients. Prior to the procedure, the patient should be assessed for blood hemoglobin and platelet levels, and prothrombin time.

A liver biopsy may not be necessary in patients infected with HCV genotypes 2 and 3, as over 80% of these patients achieve a sustained virological response to standard treatment. It is unclear, whether a biopsy is required in patients infected with HCV genotype 1 (88). Consequently, the decision as to whether a liver biopsy is needed should be based on whether treatment is being considered. Also, physicians should take

into account the estimated duration of infection, indices of advancing liver disease, the viral genotype, and the patient's willingness to be biopsied and receive treatment.

1.8. Treatment

1.8.1. Objectives and outcomes

The goal of therapy is to prevent complications and death from HCV infection. Because of the slow evolution of chronic HCV infection over several decades, it has been difficult to demonstrate that therapy prevents complications of liver disease. Accordingly, treatment responses are defined by a surrogate virological parameter rather than a clinical endpoint. Short-term outcomes can be measured biochemically (normalization of serum ALT levels), virologically (absence of HCV RNA from serum by a sensitive PCR-based assay), and histologically (<2 point improvement in necroinflammatory score with no worsening in fibrosis score). Several types of virological responses may occur, labeled according to their timing relative to treatment **(Table 4.)**. The most important is the sustained virological response (SVR), defined as the absence of HCV RNA from serum by a sensitive PCR assay 24 weeks following discontinuation of therapy. This is generally regarded as a "virological cure," although liver cancer has been identified years later, especially if cirrhosis existed at the time of achieving an SVR (89).

1.8.2. Current standard of therapy

The currently recommended therapy of chronic HCV infection is the combination of a pegylated interferon alfa and ribavirin. There are two licensed pegylated interferons: peginterferon alfa-2b (Peg-Intron, Schering Plough Corp., Kenilworth, NJ), with a 12-kd linear polyethylene glycol (PEG) covalently linked to the standard interferon alfa-2b molecule, and peginterferon alfa-2a (Pegasys, Hoffmann-La Roche, Nutley, NJ) with a 40-kd branched PEG covalently linked to the standard interferon alfa-2a molecule.

The doses of these two forms of pegylated interferons differ. The optimal dose of peginterferon alfa-2b is 1.5 μg/kg/week dosed according to body weight. Although the dose of ribavirin used in the original registration trial was fixed at 800 mg daily, a subsequent community-based study of patients with genotype 1 infection demonstrated

Virological Response	Definition	Clinical Utility
Rapid virological response (RVR)	HCV RNA negative at treatment week 4 by a sensitive PCR-based quantitative assay	May allow shortening of course for genotypes 2&3 and possibly genotype 1 with low viral load
Early virological response (EVR)	≥2 log reduction in HCV RNA level compared to baseline HCV RNA level (partial EVR) or HCV RNA negative at treatment week 12 (complete EVR)	Predicts lack of SVR
End-of-treatment response (ETR)	HCV RNA negative by a sensitive test at the end of 24 or 48 weeks of treatment	
Sustained virological response (SVR)	HCV RNA negative 24 weeks after cessation of treatment	Best predictor of a long-term response to treatment
Breakthrough	Reappearance of HCV RNA in serum while still on therapy	
Relapse	Reappearance of HCV RNA in serum after therapy is discontinued	
Nonresponder	Failure to clear HCV RNA from serum after 24 weeks of therapy	
Null responder	Failure to decrease HCV RNA by < 2 logs after 24 week of therapy	
Partial responder	Two log decrease in HCV RNA but still HCV RNA positive at week 24	

Table 4. Virological Responses During Therapy and Definitions

From Ghany et al. (88)

that weight-based ribavirin (800 mg for patients <65 kg; 1,000 mg for patients weighing 65 to 85 kg; 1,200 mg for patients weighing 85 to 105 kg; and 1,400 mg for patients weighing >105 kg but <125 kg) was more effective (90).

Peginterferon alfa-2a is administered at a fixed dose of 180 μg/week given subcutaneously together with ribavirin 1,000 to 1,200 mg daily, 1,000 mg for those who weigh ≤75 kg and 1,200 mg for those who weigh >75 kg (91). The optimal duration of treatment should be based on the viral genotype. Randomized trials established that patients with genotype 1 should be treated for 48 weeks with peginterferon alfa-2a plus standard weight-based ribavirin, whereas patients with genotypes 2 and 3 could be treated with peginterferon alfa-2a plus low dose ribavirin (800 mg) for 24 weeks. For patients with HCV genotype 4 infection, combination treatment with pegylated interferon plus weight-based ribavirin administered for 48 weeks appears to be the optimal regimen, as concluded in a meta-analysis of six randomized trials (92).

1.8.3. Pretreatment predictors of response

Multivariate analyses have identified two major predictors of an SVR among all populations studied: the viral genotype and pretreatment viral load (91, 93). Sustained virological response rates were higher in patients infected with genotype non-1 infection (mostly genotype 2 and 3) and in those with a viral load of less than 600,000 IU/mL (94). Other less consistently reported baseline characteristics associated with a favorable response include the doses of peginterferon (1.5 μg/kg/week versus 0.5 μg/kg/week) and ribavirin (>10.6 mg/kg), female gender, age less than 40 years, non-African-American race, lower body weight (≤75 kg), the absence of insulin resistance, elevated ALT levels (three-fold higher than the upper limit of normal), and the absence of bridging fibrosis or cirrhosis on liver biopsy (88).

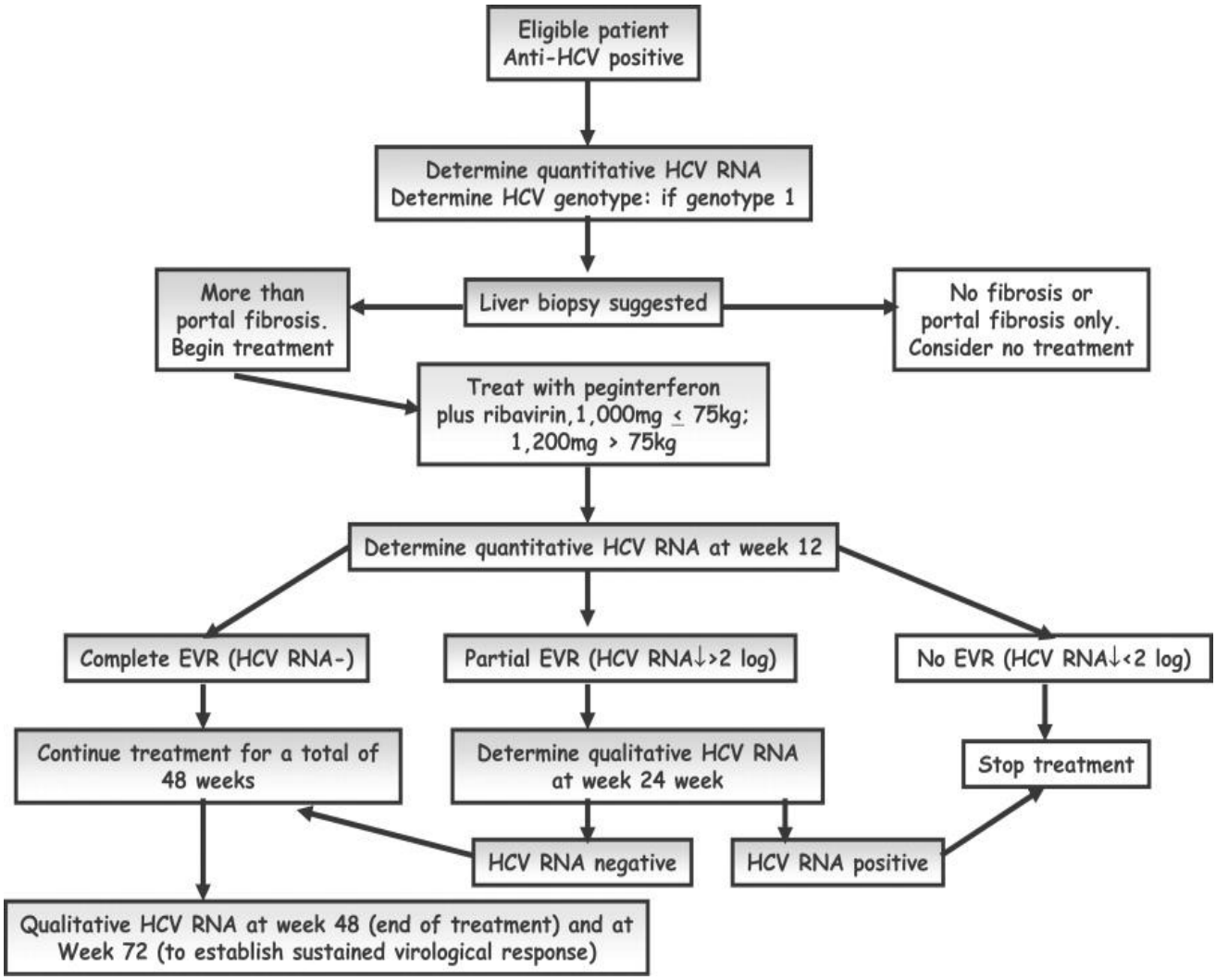


Figure 8. Treatment algorithm for managing and treating patients with chronic HCV infection, genotype 1

SVR, sustained virologic response; EVR, early virologic response.

From Ghany et al. (88)

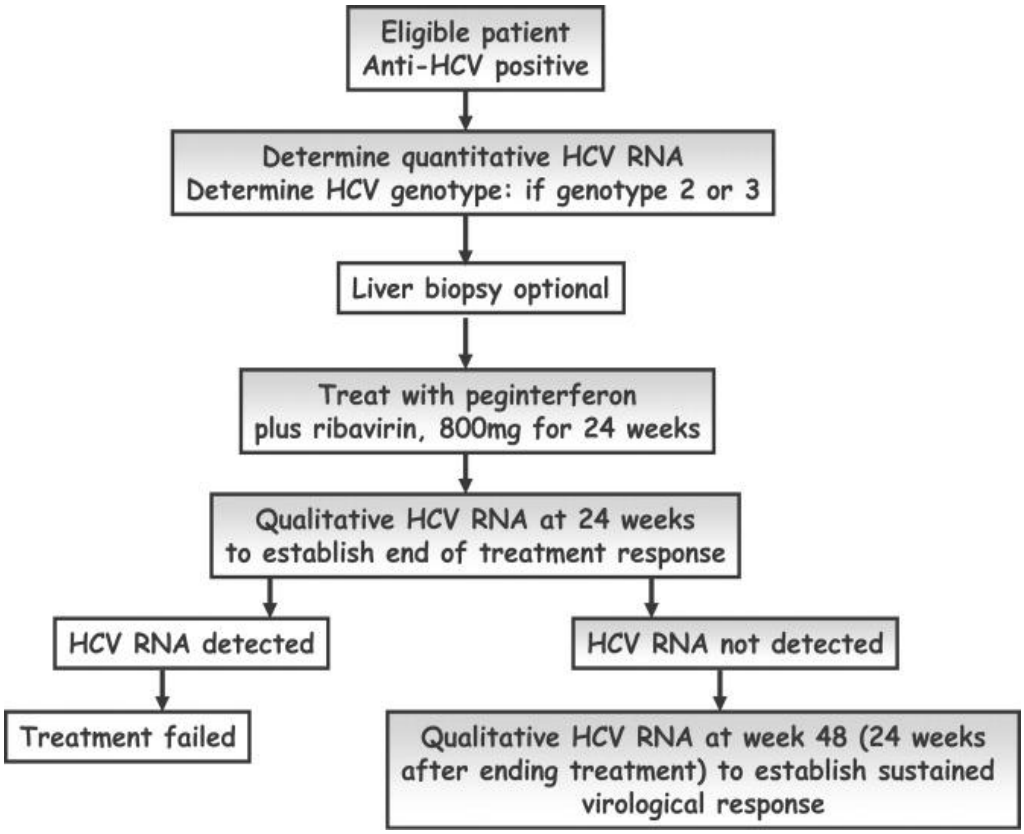


Figure 9. Treatment algorithm for managing and treating patients with chronic HCV infection, genotype 2 or 3

EVR, early virologic response; ETR, end of treatment response; SVR, sustained virologic response.

From Ghany et al.(88)

The sequential steps recommended for managing and treating persons chronically infected with hepatitis C are summarized in **Figure 8 and 9**, according to the currently acceptable guidelines (88). However, it is recognized that reasonable physicians may deviate from the strategy and remain within acceptable standards of treatment.

1.9. Extrahepatic manifestations

According to the numerous studies and case reports, 40–74% of patients may develop at least one extrahepatic manifestation during the course of the disease. The significance of extrahepatic manifestations is multiple: one hand, it may be associated with more severe symptoms than hepatic disease itself, causing severe complaints and impaired quality of life in patients. On the other hand, extrahepatic manifestations can represent the first clinical manifestation of HCV infection, even in the absence of hepatic symptoms, thus becoming the major hint during the diagnosis of the disease (95). Further, the infected extrahepatic tissues might act as a reservoir for HCV and play a role in both HCV persistence and reactivation of infection (96).

At least 36 extrahepatic disease manifestations, mainly autoimmune disorders, have been reported to be associated with HCV infection **(Table 5.)** (97). The true prevalence of any of these manifestations is not known because studies have not been performed on large numbers of unselected HCV-infected patient populations.

Mixed cryoglobulinemia is the most known and studied syndrome in HCV infection. Cryoglobulins are immunoglobulins that precipitate at temperatures below 37°C, leading to systemic vasculitis characterized by deposition of circulating immune complexes in blood vessels of small and medium size. Cryoglobulins have been classified on the basis of the immunoglobulin clonality (**Table 6.)** (98). HCV is strongly associated with cryoglobulin type II and III. Cryoprecipitates usually contain large amounts of HCV antigen and/or antibodies against HCV. Cryoglobulins are present in up to 50% of HCV-infected patients, although less than 15% have symptomatic disease (99). An independent association has been described between cryoglobulinemia and steatosis, and advanced fibrosis (100). Clinical features of mixed cryoglobulinemia include cutaneous vasculitis, membranous proliferative glomerulonephritis, and peripheral neuropathy.

Antiphospholipid syndrome	MALToma
Aplastic anemia	Membranoproliferative glomerulonephritis
Autoimmune hemolytic anemia	Membranous glomerulonephritis
Autoimmune thyroiditis	Mixed cryoglobulinemia
Behcet's syndrome	Mooren corneal ulcers
Carotid atherosclerosis	Multiple myeloma
CREST syndrome	Non-Hodgkin's lymphoma
Dermatomyositis	Neurocognitive impairment
Diabetes mellitus	Pancreatitis
Fatigue syndrome	Polyarteritis nodosa
Fibromyalgia	Polymyositis
Guillain-Barré syndrome	Porphyria cutanea tarda
Hypertrophic cardiomyopathy	Rheumatoid arthritis
Hypocholesterolaemia	Sialadenitis
Idiopathic pulmonary fibrosis	Sjögren's syndrome
Idiopathic thrombocytopenia purpura	Systemic lupus erythematosus
IgA deficiency	Uveitis
Lichen planus	Waldenstrom's macroglobulinemia

Table 5. Reported extrahepatic disease associations with HCV infection

From Agnello et al. (97)

Type	Clonality of immunoglobulins	Associated diseases
Type I	Monoclonal immunoglobulins (IgG or IgM)	Lymphoproliferative diseases
Type II (mixed)	Polyclonal immunoglobulins (mainly IgG) plus monoclonal immunoglobulins (IgM, IgG, IgA)	Mixed cryoglobulinemia
Type III (mixed)	Polyclonal IgG and polyclonal IgM	Mixed cryoglobulinemia

Table 6. Classification of cryoglobulins

From Gallosi et al. (95)

1.10. Neurological manifestations

Among extrahepatic manifestations, neurologic complications are known to involve the peripheral or the central nervous system, leading to mild to severe symptoms and complaints. Thus, HCV infection should be considered in the differential diagnosis of a variety of neurologic disorders.

1.10.1. Peripheral nervous system manifestations

Evidence from several studies indicates that peripheral neuropathy is the commonest and best established neurologic complication of HCV infection. The prevalence of peripheral neuropathy in patients with HCV varies from 8% to 10.6% (101). Clinically, the neuropathy presents as a distal symmetric sensorimotor polyneuropathy, mononeuropathy multiplex, or mononeuropathy. It can be subacute, chronic, or acute on chronic, although a subacute onset is most common. The disorder often begins with asymmetric sensory symptoms such as paresthesias that later become symmetric. Motor symptoms appear later in the course and usually involve lower limbs more than upper limbs. Central nervous system manifestations may accompany the neuropathy. Painful palpable purpura due to a leukocytoclastic vasculitis is often seen in

the legs in a distal greater than proximal distribution. Recently, cases of polyneuropathy with cryoglobulinemia, pure motor axonal neuropathy, small fiber neuropathy presenting with restless legs syndrome, and cranial neuropathy have also been described (102).

The type and severity of neuropathy may depend on the presumed pathogenetic mechanism. Cryoglobulin positive patients are presented more frequently with moderate-to-severe polyneuropathy, while cryoglobulin-negative patients tend to present with mild-to-moderate mononeuropathy or mononeuropathy multiplex (103). Patients with PAN-type vasculitis are more likely to have a severe, acute, sensorimotor mononeuropathy multiplex involving all extremities. A demyelinating polyneuropathy may be related to the effects of HCV on lymphocyte proliferation (104). Neuropathologic findings in HCV neuropathy include axonal degeneration, differential fascicular loss of axons, signs of demyelination, and small-vessel vasculitis with mononuclear cell infiltrates in the perivascular area (105). Altogether, further research is needed to examine the relationship between HCV infection, immunologic and pathologic changes, and the resulting form of neuropathy.

1.10.2. Central nervous system manifestations

Fatigue and depression are common complaints among HCV-infected patients, but these symptoms are also common among the general population and, in HCV-infected patients, are likely to be due to multiple, coexistent causes. The contribution of a biologic effect of HCV on cerebral function resulting in these symptoms is unclear.

Cognitive impairment has also been reported. HCV-infected patients have been found to perform worse on neuropsychologic tasks compared with control populations, after a number of other potential causes, such as substance abuse, depression, medications, liver cirrhosis, metabolic disorders, and neurologic disorders, were excluded (106). However, a definitive link between cognitive impairment and HCV infection has not been established.

Stroke can be the first extrahepatic manifestation of HCV infection. Both ischemic and hemorrhagic strokes have been described. The reported patients were relatively young and did not have hypertension or other risk factors for stroke. Strokes

were typically subcortical, involving the cerebral white matter. Imaging studies of patients with stroke and HCV infection showed periventricular or more extensive white matter lesions (101). Strokes related to HCV infection are usually associated with the presence of cryoglobulinemia and vasculitis, further, direct effect of the virus on the brain has also been proposed, based on the detection of HCV genomic sequences in the cerebrospinal fluid CSF of HCV/HIV-infected patients (107).

1.10.3. Autonomic nervous system manifestations

Autonomic system function in patients with chronic hepatitis C virus infection has not been studied before. However, presence and clinical importance of cardiovascular autonomic dysfunction in chronic liver diseases has been examined and proved by several studies.

Autonomic dysfunction has been described in both chronic alcoholic and non-alcoholic liver diseases, including primary biliary cirrhosis and chronic hepatitis B virus infection, as well as in patients with different liver stages of histology ranging from moderate liver fibrosis to cirrhosis (108,109,110,111). Besides, autonomic neuropathy represents a serious complication as it carries a 5-fold risk of mortality within 4 years, in patients with chronic liver diseases independent from the severity of the liver disease (112). In a 10-month-long follow-up study in patients awaiting for liver transplantation the mortality was significantly higher in patients with autonomic neuropathy (27%) compared to those without it (0%), suggesting that presence of neuropathy should be taken into consideration for early liver transplantation in patients with advanced liver disease (113).

Up to now the precise explanation of increased mortality associated with autonomic neuropathy in patients with chronic liver diseases has not been clearly identified. Beside the most severe complications of autonomic dysfunction - silent myocardial ischemia and infarction, cardiorespiratory arrest, major arrhythmias (114) - the attenuation of circadian variation of blood pressure and heart rate may contribute to the higher death rate (115, 116). Autonomic neuropathy may also be regarded as a potential etiologic factor of hyperdynamic circulation and portal hypertension (117).

1.11. Determination of cardiovagal autonomic function: heart rate variability and baroreflex senxitivity

Since the recognition of a significant relationship between the autonomic nervous system and cardiovascular mortality, numerous experimental methods have been used to describe the quantitative markers of autonomic activity. Heart rate variability (HRV) represents one of the most promising such markers. The easy derivation of this measure has popularized its use. As many commercial devices now provide automated measurement of HRV, providing a simple non-invasive tool for both research and clinical studies (118).

Variations in heart rate may be evaluated by a number of methods. The simplest to perform are the time domain measures. Various time-domain indices can be calculated, including include the standard deviation of all RR intervals (NNSD), the mean heart rate, the difference between the longest and shortest RR interval, the difference between night and day heart rate, means the root mean square of successive RR-interval differences (RMSSD), means the percentage of RR intervals that differ >50 ms (pNN50) etc.; however, many of the measures correlate closely with others.

Power spectral density analysis provides the basic information of how power distributes as a function of frequency. Methods for the calculation of spectral density may be generally classified as non-parametric and parametric. In most instances, both methods provide comparable results. Advantages of non-parametric methods are the simplicity of the algorithm employed (fast Fourier Transformation) and the high processing speed. Three main spectral components are distinguished in a spectrum calculated from short-term recordings of 2 to 10 min: power in very low frequency range (≤0.04 Hz) (VLF), power in low frequency range (0.04-0.15 Hz) (LF), and power in high frequency range (0.15-0.4 Hz) (HF) components. VLF assessed from short-term recordings is a dubious measure and should be avoided when interpreting the power spectral density of short-term ECGs. According to some study groups, spectral indices are able to discriminate between sympathetic and parasympathetic contributions, e.g. vagal activity is the major contributor to the HF component, while LF reflects rather sympathetic activity. Although this conclusion has theoretical background, the

possibility of determining sympathetic/parasympathetic balance this way remains under strong debate (122).

Besides HRV, another widely used method to quantify autonomic activity is to determine the baroreceptor reflex sensitivity (BRS). Baroreflex is probably one of the most important cardiovascular control mechanisms adjusting heart rate and sympathetic output to the blood vessels on a beat-by-beat basis. Like many other physiologic feedback control mechanisms, baroreflex control of the heart rate can be modeled as a sigmoidal stimulus (systolic blood pressure) – response (R-R interval) curve with a threshold point, a saturation point, and a linear relationship in-between. The effect of a functioning baroreflex results in a dampening of systolic blood pressure increase because of an increase of the corresponding R-R intervals and vice versa. Calculation of BRS can be obtained by sequence method, as the slope of the linear regression lines between the R-R intervals and the systolic blood pressure values, reflecting the sensitivity (gain) of the baroreflex in ms/mmHg. Similarly to HRV analyses, power spectral densities of BRS can be calculated, as well, using fast Fourier Transformation. The transfer function gains can be defined for the high frequency (0.15– 0.5 Hz) and low frequency bands (0.05–0.15 Hz). The alpha coefficients is calculated as the square root of the mean spectral density of the R-R interval spectra divided by the mean density of the systolic blood pressure spectra for the two frequency bands, separately (123).

2. Aims

The aims of my studies were to describe cardiovagal autonomic function in patients with chronic hepatitis C virus infection, and to examine the possible changes of autonomic function during the current antiviral therapy.

I aimed to answer the following questions:

1. Is there a difference in cardiovagal autonomic function between treatment-naïve patients with chronic HCV infection and healthy control people?

2. Is there a correlation between autonomic indices and markers of liver cell damage (serum aminotransferases), liver synthetic capacity (serum albumin), glucose metabolism, cryoglobulins and serum HCV RNA level in patients with chronic HCV infection?

3. How does the current standard of antiviral therapy affect the cardiovagal autonomic function during the course of the treatment?

4. Is there an association between with autonomic function indices and the anthropometric and laboratory variables (age, BMI, cryoglobulinemia, HCV RNA level, ALT, albumin, glucose) and the response to therapy during antiviral treatment?

3. Methods

3.1. Patients and controls

For the cross-sectional study, forty-five patients with chronic HCV infection (range 26–62 years of age, mean 48.2) were recruited from three outpatient liver clinics in Budapest, Hungary, between January 2006 and December 2007. Forty healthy subjects (range 28–67 years of age, mean 44.6) were recruited from the medical and assistant staff of different medical departments of Semmelweis University and served as controls. Inclusion criteria only for patients were: (i) HCV RNA positivity by polymerase chain reaction (PCR); (ii) no previous antiviral treatment; and (iii) no liver disease in history other than chronic HCV infection. Inclusion criteria for both patients and controls were: (i) no histological, laboratory or clinical evidence of liver cirrhosis; (ii) no disease that might affect the autonomic nervous system, such as diabetes, hypertension, heart failure, ischemic heart disease, end-stage renal disease, stroke, Parkinson's disease, AIDS; (iii) no treatment influencing autonomic nervous system such as beta blockers, muscarinic receptor blockers; (iv) no alcohol or drug abuse; and (v) sinus rhythm.

For the follow-up study, twenty-two patients with chronic HCV infection were recruited from the same three outpatient liver clinics in Budapest, between January 2006 and December 2007. Inclusion criteria were the same as listed in the cross-sectional study. For the start of the antiviral therapy, additional contraindications were the following: white blood cell count <3,000/mm^3, absolute neutrophil count <1,500/mm^3, and platelet count <80,000/mm^3 as well as uncertainty about effective contraception during therapy and uncontrolled psychiatric condition, pregnancy or lactation.

All individuals gave written informed consent to participate in the studies that were approved by the Ethics Committee of the Semmelweis University, Budapest, Hungary.

3.2. Serological studies

In 1 day before autonomic function tests the following routine laboratory analyses were performed: aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin and blood glucose. In patients with HCV infection, HCV RNA was quantified by real-

time PCR (COBAS TaqMan, Roche Diagnostics, Meylan, France). Genotyping was performed by reverse hybridization (INNO-LiPA HCV II, Innogenetics, Ghent, Belgium). For the detection of mixed cryoglobulins, blood samples were kept at 37 °C until complete coagulation and were analyzed by standard methods.

3.3. Cardiovagal autonomic function

3.3.1. Blood pressure, heart rate and respiration

Radial artery pressure was monitored continuously with an automated tonometric device (Colin CBM-7000; AD Instruments) for determination of BRS indices. Systolic (SBP) and diastolic blood pressure (DBP) measured on the brachial artery by an automatic microphonic sphygmomanometer built into the Colin device were used to calibrate the radial pressure pulse. R-R intervals (RRI) were measured from R-wave threshold crossings on continuously recorded ECGs. Respiration was recorded with an inductive system (Respitrace System; Ambulatory Monitoring). To improve the reliability of the measurements, breathing rate was paced at 0.25 Hz (119).

3.3.2. Baroreflex sensitivity (BRS)

The coupling between spontaneous fluctuations in heart rate and SBP was determined by the sequence method and by spectral analysis. The software used (WinCPRS program; Absolute Aliens Oy) detected the ECG R wave, computed RRI and radial artery SBP time series and identified spontaneously occurring sequences in which SBP and RRI concurrently increased and decreased over three or more consecutive beats (BRS_{seq}). The minimal accepted change was 1 mmHg for SBP and 5 ms for RRI. Only sequences with a correlation coefficient >0.85 were considered. To determine spectral indices, the signals were interpolated, resampled and their power spectra were determined using Fast Fourier Transformation-based methods. The LF_{gain} (LF [low-frequency] transfer function gain) was determined, which expresses RRI and SBP cross-spectral magnitude in the frequency range of 0.05–0.15 Hz, where coherence is greater than 0.5.

3.3.3. Heart rate variability (HRV)

Time and frequency domain measurements of HRV from 10 min recordings of RRIs were calculated using the WinCPRS program. Non-sinus beats were semi-automatically removed and corrected using interpolation of preceding beats. The following parameters were determined: the standard deviation of the RRI (termed NNSD), the root-mean-square of successive differences (termed RMSSD), the percentage of successive RRIs which differed by more than 50 ms (termed pNN50), as well as low frequency (0.05–0.15 Hz) and high-frequency (0.15–0.4 Hz) power of RRI variability (termed LF and HF, respectively).

3.3.4. Examination of cardiovagal autonomic function

Patients and controls were studied in the early afternoon under standardized conditions, in a quiet room at a comfortable temperature, in the research laboratory of the Institute of Human Physiology and Clinical Experimental Research, Semmelweis University, Budapest, Hungary. All individuals fasted at least 2 hours before testing and were asked to refrain from strenuous exercise or drinking alcohol or caffeinated beverages for 24 hours prior to the study. Subjects were equipped with the appropriate devices, and then rested in supine position for approx. 15 minutes until baseline conditions for heart rate and mean blood pressure were reached. Subjects were then asked to synchronize their respiratory rate with a metronome beating at 0.25 Hz. R-R intervals and radial artery pressure were recorded continuously for a 10 minute period to determine spontaneous baroreflex indices.

3.4. Study design

For the cross-sectional study, patients and controls underwent laboratory examinations and cardiovagal autonomic function assessment once, with the above mentioned methods.

For the follow-up examinations, patients were treated with pegylated interferon (PEG-IFN) alfa-2a (40kd, 180μg) or PEG-IFN alfa-2b (12kd, 1.5 μg/kg) subcutaneously once per week and ribavirin (1000 mg if < 75 kg and 1200 mg if >75 kg) per os daily.

Duration of therapy was considered individually according to the guidelines. Treatment responses were characterized by the results of HCV RNA testing. Early virologic response was defined as a decrease in serum HCV RNA concentration to <50 IU/mL or a decrease of at least 2 log units from baseline viral load at week 12 of therapy. SVR was defined as negativity for HCV RNA in serum by a PCR test at the end of treatment and 6 months later. Autonomic function and laboratory examinations were performed one day before therapy, then on week 12, week 24 and week 48, depending on the individualized duration of antiviral therapy.

3.5. Data analysis

Blood pressure and ECG recordings were digitized and analyzed using the WinCPRS program using a sampling rate of 500 Hz. Data were expressed as means ± S.D.

Between controls and patients, differences in variables were analyzed using unpaired Student's *t* tests or Mann–Whitney rank-sum for data that failed the tests of normality. To assess the independent effect of different parameters (age, body mass index [BMI], SBP, DBP, serum levels of AST, ALT, albumin, glucose, cryoglobulinemia, HCV RNA) on autonomic indices (HRV and BRS), we performed a multivariate analysis using full information maximum likelihood regression. To correct for any violations of residual normality, robust standard errors were used. Serum HCV RNA level was logged to correct its heavily skewed distribution.

To assess the change over time in variables of interest, repeated variance analysis was used with post hoc Tukey tests. To ensure compliance with the assumptions of repeated measures ANOVA requiring equal interval measures, two independent analyses were conducted: one with values before and at week 12 and 24 of treatment; and one with values before and at week 24 and 48 of treatment. To evaluate the effect of potential determinants (age, body mass index [BMI], serum levels of ALT, albumin, glucose, cryoglobulinemia, HCV RNA, and response to therapy) on autonomic (HRV and BRS) indices during therapy, multivariate analysis using full information maximum likelihood regression was performed including all selected participants and correcting for attrition.

Significance was accepted at $p<0.05$. Statistical analyses were performed using SPSS program package version 14 (SPSS Inc., Chicago, IL, USA) and Mplus version 5.2 (Muthen and Muthen, Los Angeles, CA, USA).

4. Results

4.1. Results of the cross-sectional study

4.1.1. Clinical characteristics of study cohort

Anthropometric, hemodynamic variables and laboratory data of patients with chronic HCV infection and controls are presented in **Table 7.** There was no difference in BMI, blood pressure, heart rate, serum albumin and glucose levels between patients and controls, while AST and ALT levels were significantly higher in patients with chronic HCV infection ($p<0.001$). In patients with chronic HCV infection, the mean serum HCV RNA level was 2,400,000 IU/ml (range 5,800–10,700,000). Genotype analyses proved HCV subtype 1b in 43/45 patients (95.6%), and subtype 1a in 2/45 patients (4.4%). Mixed cryoglobulinemia was present in 11/45 patients (24%).

4.1.2. Autonomic function indices

As shown in **Table 8.**, heart rate variability sequence indices (NNSD, RMSSD, pNN50), frequency-domain indices (LF and HF), baroreflex sensitivity sequence index (BRS_{seq}) and frequency-domain index (LF_{gain}) were lower in patients with HCV infection compared to controls ($p<0.01$).

4.1.3. Associations between autonomic function and clinical characteristics

Table 9. shows the possible independent associations between autonomic function indices and the ten examined clinical variables in patients with chronic HCV infection.

Multivariate analysis proved that neither anthropometric (age, BMI), nor hemodynamic (SBP, DBP), nor specific serum variables (serum HCV RNA, glucose, albumin level, cryoglobulinemia) were independently associated with any of the examined autonomic function indices. ALT was the only parameter independently associated with RMSSD, pNN50 and HF [standardized coefficient (β) = -0.58; -0.649; -0.642, respectively, $p<0.05$]. Conducting the same analysis in controls, only age showed an independent negative correlation with RMSSD, pNN50, LF, HF, BRS_{seq} and LF_{gain} **(Table 10.)**.

	Patients	**Controls**
Subjects (male)	45 (20)	40 (16)
Age (years)	48.2 ± 8.4	44.6 ± 10.8
BMI (kg/m^2)	26.3 ± 4.1	26.4 ± 4.8
SBP (mmHg)	122.7 ± 12.8	120.2 ± 11.1
DBP (mmHg)	75.7 ± 7.9	73.9 ± 8.4
Heart rate (beats/min)	75.7 ± 9.9	72.8 ± 9.8
AST (IU/l)	94.2 ± 58.6 *****	20.1 ± 6.2
ALT (IU/l)	108.9 ± 67.4 *****	20.5 ± 9.2
Albumin (g/l)	46.4 ± 7.3	46.3 ± 2.9
Glucose (mmol/l)	5.4 ± 0.6	5.2 ± 0.6

Table 7. Clinical characteristics of study cohort

Values are given as means ± SD. BMI indicates body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; AST, aspartate aminotransferase and ALT, alanine aminotransferase. ***** $p< 0.001$, compared with controls.

	Patients	**Controls**
NNSD (ms)	30.5 ± 10.7 *****	42.7 ± 19.2
RMSSD (ms)	21.7 ± 12.2 *****	32.9 ± 22.7
pNN50 (%)	4.4 ± 6.8 *****	13.4 ± 18.0
LF (ms^2)	168.5 ± 160.9 *****	370.7 ± 349.4
HF (ms^2)	182.6 ± 198.1 *****	388.9 ± 361.3
BRS_{seq} (ms/mmHg)	7.1 ± 3.4 *****	11.5 ± 6.5
LF_{gain} (ms/mmHg)	5.2 ± 3.3 *****	7.9 ± 5.3

Table 8. Cardiovagal autonomic function indices of study cohort

Values are given as means ± SD. NNSD indicates SD of RR intervals; RMSSD, root mean square of successive RR-interval differences; pNN50, percentage of RR intervals that differ >50 ms; LF, low-frequency (0.05–0.15 Hz) power of RR-interval variability; HF, high-frequency (0.15–0.4 Hz) power of RR-interval variability. BRS_{seq} indicates baroreflex sensitivity sequence index; LF_{gain}, cross-spectral transfer gain in the low-frequency range. ***** $p < 0.01$, compared with controls.

	NNSD	RMSSD	pNN50	LF	HF	BRS_{seq}	LF_{gain}
Age	-0.098	-0.021	-0.102	-0.09	-0.161	-0.223	-0.247
BMI	-0.114	-0.189	-0.082	-0.021	-0.125	-0.062	-0.048
SBP	-0.155	0.029	-0.342	-0.051	-0.3	-0.148	-0.274
DBP	0.006	-0.181	0.293	0.052	0.166	0.046	0.264
AST	-0.009	0.124	0.338	-0.042	0.314	-0.087	0.004
ALT	-0.297	-0.58*	-0.649*	-0.147	-0.642*	-0.225	-0.232
Albumin	0.254	0.225	0.248	-0.02	0.287	0.227	0.263
Glucose	-0.133	-0.214	-0.181	-0.017	-0.174	0.047	-0.023
Cryoglobulins	0.207	0.151	0.169	-0.03	0.212	0.023	0.052
HCV-RNA	0.001	0.105	0.148	-0.002	0.057	0.061	-0.085
r^2	0.293	0.426	0.339	0.067	0.381	0.285	0.265

Table 9. Independent effects on autonomic function variables in patients with chronic HCV infection

Multivariate analysis was performed using full information maximum likelihood regression. Standardized coefficients are given. ***** $p < 0.05$

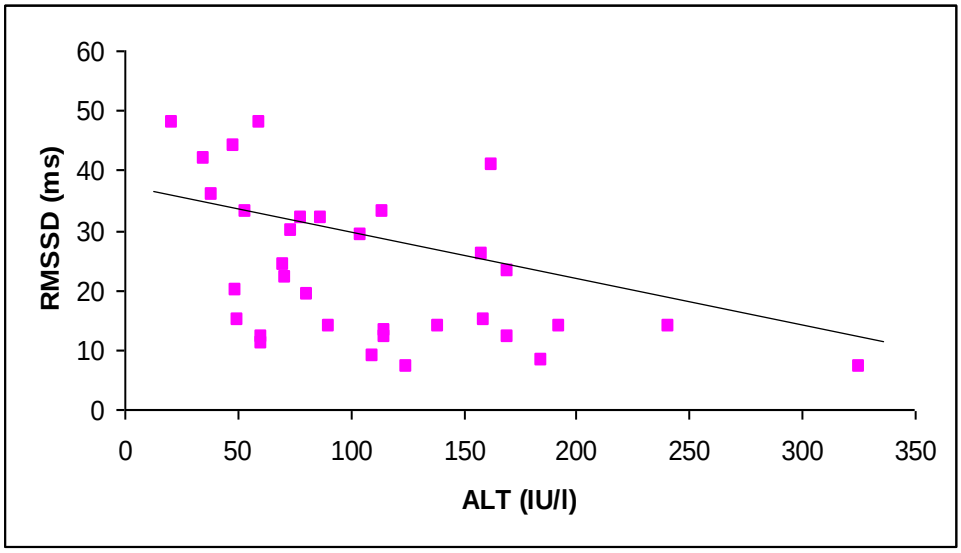


Figure 10. Alanine aminotransferase (ALT) - related changes in root mean square of successive R-R interval differences (RMSSD)

r=-0.58, $p< 0.05$

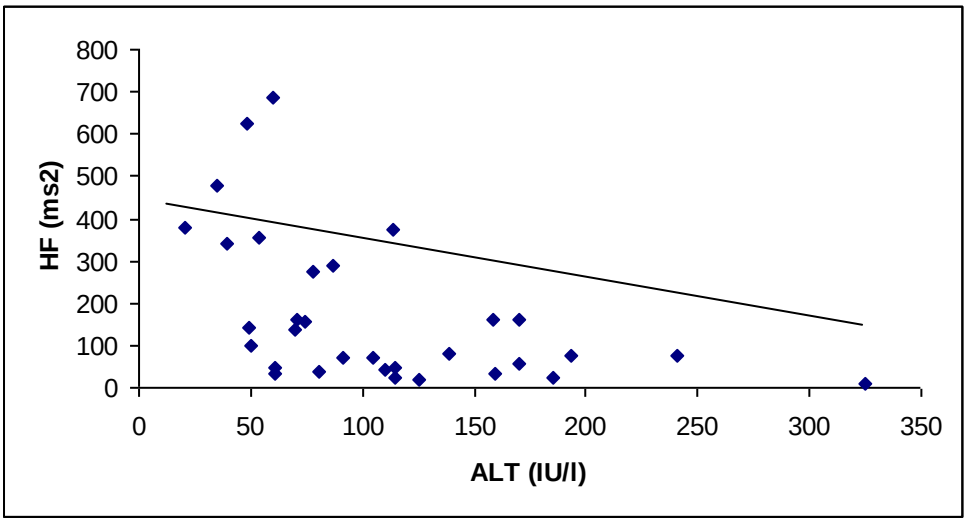


Figure 11. Alanine aminotransferase (ALT) - related changes in high frequency domain indices (HF) of heart rate variability

r=-0.642, $p< 0.05$

	NNSD	RMSSD	pNN50	LF	HF	BRS_{seq}	LF_{gain}
Age	-0.21	-0.291**	-0.259*	-0.343**	-0.308*	-0.311**	-0.313*
BMI	-0.101	-0.182	-0.131	-0.109	-0.092	-0.007	0.059
SBP	-0.167	0.081	-0.081	-0.081	-0.052	-0.014	-0.251
DBP	-0.009	-0.27	-0.034	0.141	0.108	0.015	-0.028
AST	-0.406	-0.223	-0.153	-0.388	-0.222	-0.296	-0.331
ALT	0.365	0.197	0.158	0.231	0.181	0.16	0.085
Albumin	-0.189	-0.203	-0.236	-0.148	-0.114	-0.01	-0.001
Glucose	-0.063	-0.068	-0.08	-0.17	-0.161	-0.209	0.076
r^2	0.29	0.271	0.219	0.408	0.257	0.29	0.255

Table 10. Independent effects on autonomic function variables in healthy controls
Multivariate analysis was performed using full information maximum likelihood regression. Standardized coefficients are given. ***p*< 0.05, ****p*< 0.01

4.2. Results of the follow-up study

Twenty-two patients were eligible and started antiviral treatment (age=44.9 ± 10.1; 26-62 years, BMI=26.1 ± 5.4; 19.8-40.4 kg/m^2, means ± SD; range). Mean serum HCV RNA level was 1,900,000 IU/ml (range 5,800–10,700,000), genotype analyses proved HCV subtype 1b in 21/22 patients (95%), and subtype 1a in 1/22 patients (5%). Mixed cryoglobulinemia was present in 8/22 patients (35%) before treatment. Study and treatment protocol was discontinued in one patient in week 14 because of severe fatigue, one patient died in traffic accident in week 31, and one patient refused study examinations in week 48. Among the 22 patients 15 (68%) showed an early virological response at week 4 or 12, and 11/19 (58%) patients achieved a sustained virological response (SVR).

The patients' laboratory data and cardiovagal autonomic indices before and during antiviral treatment are given in **Table 11.** To preserve space, results of the two ANOVA analyses were combined for presentation in the same table. The initially elevated AST/ALT levels were nearly normalized by week 12 of therapy and remained normal throughout weeks 24 and 48 ($p< 0.001$). Glucose or albumin levels remained unchanged before and during therapy. Both heart rate variability sequence indices (NNSD, pNN50), frequency-domain indices (LF and HF), and baroreflex sensitivity indices (BRS_{seq}, LF_{gain}) significantly decreased by week 12 ($p< 0.01$), and then increased by week 24 ($p< 0.05$) to reach pre-treatment levels by week 48 of antiviral therapy.

Multivariate analyses did not identify a significant correlation between any of the anthropometric or laboratory variables (age, BMI, cryoglobulinemia, HCV RNA level, ALT, albumin, glucose) and changes of autonomic function indices during antiviral treatment. Response to therapy was also not associated with the changes of autonomic function.

	Before treatment	Treatment week 12	Treatment week 24	Treatment week 48
n (male)	22 (11)	22 (11)	21 (11)	19 (10)
AST (IU/l)	92.1 ± 56.2	45.7 ± 27.7 *	39.5 ± 26.7 *	42.16 ± 23.8*
ALT (IU/l)	113.3 ± 71.5	37.8 ± 26.1 *	33.2 ± 27.8 *	33.7 ± 21.8*
Albumin (g/l)	45.8 ± 7.1	43.8 ± 4.3	45.6 ± 3.7	43.9 ± 4.3
Glucose (mmol/l)	5.1 ± 0.5	5.1 ± 0.7	5.1 ± 0.6	5.0 ± 0.5
cryoglobulinemia (%)	8 (35%)	5 (22%)	2 (8%)	1 (5%)
Heart rate (beats/min)	73.1 ± 6.0	76.2 ± 7.5	73.4 ± 8.1	75.2 ± 9.2
NNSD (ms)	36.8 ± 11.9	27.5 ± 10.8 †	31.3 ± 16.1	34.1 ± 11.3
RMSSD (ms)	28.0 ± 14.7	19.9 ± 13.5	29.9 ± 23.8	30.2 ± 17.3
pNN50 (%)	5.5 ± 6.2	1.4 ± 2.3 †	3.4 ± 4.4 ‡	4.4 ± 4.9
LF (ms^2)	253.0 ± 156.1	111.6 ± 81.9 †	183.4 ± 169.6 ‡	211.6 ± 149.1
HF (ms^2)	230.1 ± 165.1	105.0 ± 97.9 †	254.5 ± 333.4 ‡	251.8 ± 290.4
BRS_{seq} (ms/mmHg)	8.1 ± 3.9	5.6 ± 2.0 †	8.3 ± 4.1 ‡	8.8 ± 2.9
LF_{gain} (ms/mmHg)	6.8 ± 3.6	5.0 ± 2.2 †	5.9 ± 2.6 ‡	6.7 ± 2.4

Table 11. Laboratory data and autonomic function indices in patients with chronic hepatitis C during antiviral treatment

Values are given as means ± SD. *****Significantly different, $p< 0.001$ from "Before treatment" value; †significantly different, $p< 0.01$ from "Before treatment" value; ‡ significantly different, $p< 0.05$ from "Treatment week 12" value.

5. Discussion

In our cross-sectional study we compared cardiovagal autonomic function in treatment-naïve patients with chronic HCV infection and healthy controls. We found lower HRV and BRS indices in patients with HCV infection, indicating autonomic dysfunction. Among the clinical variables examined, serum ALT level was independently associated with HRV indices; neither cryoglobulinemia nor the serum HCV RNA level correlated with impaired autonomic function. In healthy controls, only age had an independent association with HRV and BRS indices. The negative correlation between age and autonomic function is known from other studies examining HRV and BRS variables in healthy populations (120,121). HCV subtype distribution, which reflects the known subtype distribution of Hungarian patients with HCV infection was too homogenous to study associations (**Hiba! A könyvjelző nem létezik.**).

To our knowledge, this is the first case-control study addressing autonomic function in patients with chronic HCV infection. However, autonomic function is well described and studied in other chronic liver diseases, which is reasonable for more reasons. On one hand, cardiovagal autonomic function can be assessed by non-invasive, reproducible methods: parasympathic activity, basal vagal outflow can be determined by various indices of heart rate variability (HRV), while autonomic integrative function can be estimated by measuring baroreflex sensitivity (BRS) indices (122,123). On the other hand, reduced HRV and BRS have been shown to be independent predictors of cardiovascular morbidity and mortality (124,125). Moreover, autonomic neuropathy in chronic liver disease is associated with a 5-fold increased mortality within 4 years, independent from the severity of the liver disease (**Hiba! A könyvjelző nem létezik.**). In a recent prospective study, decreased HRV indices predicted a poor prognosis and a high mortality in 30 patients with liver cirrhosis, independent from age (126). In a study including 21 patients awaiting liver transplantation, reduced HRV correlated with the severity of the liver disease (127).

Despite the numerous central and peripheral nervous system dysfunctions, that has been described in patients with HCV infection, the pathophysiology of these extrahepatic symptoms remains largely unknown. Neurological symptoms have been described in HCV infected patients in the absence of advanced liver disease, suggesting

that HCV infection may affect the nervous system directly, independent from liver function (128). In a study including 66 patients with chronic HCV infection without cirrhosis, impaired neuropsychological function was found in 49% (129). In our case-control study of patients without laboratory or clinical evidence of liver cirrhosis and no other causes of liver disease than chronic HCV infection, an independent negative correlation was found between autonomic dysfunction and serum ALT levels. In healthy controls, by comparison, only age was associated independently with autonomic function. While AST/ALT levels do not directly correlate with the severity of the liver disease (130), they indicate HCV-induced liver-cell damage. Taken together, autonomic dysfunction is not proved to correlate with the severity of the liver disease. Nevertheless, our findings suggest a common pathomechanism for hepatocellular damage and autonomic dysfunction in patients with chronic HCV infection.

Since HCV infection/replication itself is not cytopathic, both liver and neurological pathologies in chronic HCV infection are commonly considered to be immune-mediated (131,132). Based on detailed analyses including nerve biopsies, impaired vasa nervorum microcirculation due to intravascular deposits of cryoglobulins and/or immune-vasculitis have been proposed as possible pathomechanisms (133, 134).

One of the most common extrahepatic manifestations of HCV infection is the presence of serum cryoglobulins, termed essential mixed cryoglobulinemia, which is frequently found in patients with chronic HCV infection and peripheral neuropathy. In a study including 321 patients with chronic HCV infection, 50% had mixed cryoglobulinemia and 9% had clinically symptomatic sensory or motor neuropathy (135). In another study among 26 patients with chronic HCV infection and mixed cryoglobulinemia, 77% had peripheral neuropathy, as defined by the presence of paresthesias (136). In a recently published retrospective study autonomic function was examined in 30 patients with HCV-associated mixed cryoglobulinemia: 10% had autonomic dysfunction on the basis of functional cardiovascular tests (137). In our patients with chronic HCV infection, cryoglobulins were found in 24% and did not correlate with the level of AST/ALT or HCV RNA, confirming a previously published report (138). Further, there was no correlation between autonomic dysfunction and the presence of mixed cryoglobulinemia. It is known that peripheral neuropathy may also be present in patients without cryoglobulinemia, at a lower prevalence (139). This

finding suggests that, similar to our findings, cryoglobulin-independent mechanisms may be involved in the pathophysiology of these neuropathies.

Apart from immune-mediated processes, two other mechanisms underlying HCV-associated neurological manifestations have been discussed: direct nerve infection and glucose neurotoxicity. A direct axonal viral damage has been discussed since HCV RNA was detected in skin and nerve biopsy samples with vasculitis (140), as well as in muscle from patients with polymyositis (141). In a study including 30 patients with chronic HCV infection, positive-strand HCV-RNA was detected in muscle and nerve biopsies from 10 patients, while no negative-strand replicative HCV RNA was found (142). The absence of local HCV replication makes a viral contribution to the axonal damage unlikely. Further, similar to our study, Santoro et al. found no correlation between peripheral neuropathy and the level of serum HCV RNA (143).

Recently, insulin resistance has been described in HCV infection (144). Diabetic neuropathy is a well known complication of persistent episodes of hyperglycemia - a phenomenon referred to as glucose neurotoxicity (145). While diabetic patients were excluded from our study, and no differences in fasting glucose levels between patients and healthy controls were found, glucose levels did not independently correlated with indices of autonomic function in any groups. However, the determination of the fasting glucose level only is a limitation of our study, because more sophisticated analyses, such as glucose clamp technique and homeostasis model assessment (HOMA) score are required to identify insulin resistance and to assess glucose metabolism and possible effects of HCV-related insulin resistance on the development of cardiovagal autonomic dysfunction.

Another limitation of our study is the assessment of the baroreflex sensitivity, by using only the spontaneous, non-invasive method. Spontaneous BRS represents only a part of the complex neural and humoral mechanisms controlling cardiovascular function. The activation of sympathetic afferents, changes in central integration, interference with other efferent activities, and different target organ responses may directly affect BRS. Thus, spontaneous BRS values differ from the BRS values obtained in the classic way (intraarterial blood pressure measurements and pharmacological tests using phenylephrine and nitroprusside). Drug-induced changes of mean arterial pressure move the operating point into a different range, which may result in different gains. Therefore spontaneous BRS measures baroreflex sensitivity according to the actual

operating point at the response curve, which depends on the blood pressure level and the current functional state. Consequently, measurement of spontaneous BRS must be interpreted with caution, considering that the exact position of the operating point on the whole stimulus–response curve is not known (123). However, clinical studies tend to examine the spontaneous BRS, as they have primary importance to use non-invasive, fast and easy to use methods.

Following our case-control study of autonomic dysfunction in patients with chronic hepatitis C, we investigated the changes of autonomic function in patients with chronic HCV infection during antiviral therapy. Interestingly, a significant decrease in autonomic function occurred by week 12 of treatment to be followed by a significant improvement by week 24 and a return to pre-treatment values by week 48 of antiviral therapy.

The response of neurological symptoms to antiviral therapy is mostly favorable in patients with chronic hepatitis C virus infection. However, no response, worsening and even the even the first onset of peripheral neuropathy have been reported, as well (146,147,148). Further, central nervous system disturbances including depression, irritability and anxiety have been observed during antiviral treatment (149).

Ribavirin has no known neurological side effects: neither ribavirin monotherapy trials (150, 151) nor randomized trials comparing the combination of IFN-alpha and ribavirin with IFN-alpha monotherapy showed significant neurological complications (152, 153).

A direct neurotoxic effect of IFN-alpha in our study is unlikely because autonomic dysfunction improved after three months despite the continuation of therapy. However, IFN-alpha may induce vasculitis with or without cryoglobulinemia, and with its antiangiogenic activity, IFN-alpha can increase preexisting ischemia, leading to purpura, skin ulcerations, arthritis or ischemic polyneuropathy (154,155). None of these manifestations was observed in our patients with chronic HCV infection during antiviral therapy, and presence of cryoglobulins was not associated with the changes of autonomic function.

Further connection between IFN-therapy and changes of autonomic function may be the insulin resistance induced by IFN-alpha treatment, which has been described recently (156). In our study, patients with diabetes were excluded and fasting glucose levels did not change during antiviral therapy. Therefore, an impaired carbohydrate

metabolism does not underlie the changes of autonomic dysfunction in our patients. However, the fasting glucose level is insufficient to assess insulin resistance and glucose metabolism, thus, in further studies more sophisticated techniques (glucose clamp technique, homeostasis model assessment score) should be used.

The immunomodulatory effect of IFN-alpha suggests that the immune status correlates with autonomic dysfunction. Prospective longitudinal cohort studies in HIV-positive and AIDS patients indicate that autonomic dysfunction parallels the HIV disease progression., i.e, heart rate variability indices inversely correlate with the stages of immunosuppression and correlate with the CD4 cell count (157,158). The degree of autonomic dysfunction remains mild and subclinical in patients with HIV infection/AIDS in all stages of the disease, just as it was observed in our patients with chronic HCV infection. In addition, abnormal heart rate and baroreflex responses have been documented in patients with multiple sclerosis (MS) (159). In a 24-months follow-up of 26 patients with clinically active remitting- relapsing MS, autonomic dysfunction assessed by heart rate responses correlated with the clinical activity of MS and disease progression (160).

These results are consistent with data from molecular and immunological analyses, including animal-models that addressed links between dysfunction of the immune regulation and the autonomic nervous system. These studies suggest, that the balance between sympathetic/parasympathetic system plays a major role as an integrative interface between the brain and the immune system (161,162). Consistent with this hypothesis, IFN-alpha in human brain physiologically affects not only the immune but also the central nervous system. IFN-alpha and other cytokines are also involved in physiological processes such as fever or fatigue and in sympathetic/parasympathetic responses (163,164).

These findings suggest that the autonomic dysfunction in our patients with chronic HCV infection may be associated with changes of the immune status and INF-alpha modulated immune reactions. Further studies should address these interactions.

In summary, the increase of cardiovagal autonomic dysfunction during the first 12 weeks of antiviral therapy is no reason to modify the treatment strategy in patients with chronic hepatitis C virus infection because autonomic dysfunction remains mild and subclinical, and improves during continued antiviral therapy.

6. Conclusions

1. Patients with chronic HCV infection have impaired cardiovagal autonomic function, comparing to healthy controls.

2. Among the examined clinical variables, serum ALT level is the only marker independently associates with autonomic dysfunction; neither liver synthetic capacity, nor cryoglobulinemia, nor serum HCV RNA level correlates with impaired autonomic function, suggesting common pathophysiology underlying HCV-induced liver disease and neurological manifestations, through cryoglobulin-independent ways.

3. We found a significant decrease in autonomic function occurred by week 12 of treatment to be followed by a significant improvement by week 24 and a return to pre-treatment values by week 48 of antiviral therapy.

4. Multivariate analyses does not identify a significant correlation between any of the anthropometric or laboratory variables (age, BMI, cryoglobulinemia, HCV RNA level, ALT, albumin, glucose) and changes of autonomic function indices during antiviral treatment. Response to therapy is also not associated with the changes of autonomic function.

Acknowledgments

First of all, I am most grateful to my tutor, Prof. Dr. Szalay Ferenc, that he invited me to join his study group, helped me to find the research area, and gave me all his support through the years. I am especially grateful for all what I have learned from him at the patients' medical care, for the numerous medical conferences what I could attend and that I could join the student's education in internal medicine. I am grateful to my colleagues from the 1st Department of Internal Medicine, Semmelweis University for their most valuable cooperation: Dr. Gabriella Bekő, Dr. Tímea Csák, Levente Csihi, Zoltán Czermák, Dr. Anikó Folhoffer, Dr. Andrea Horváth, Dr. Evelin Horváth, Dr. Péter László Lakatos, Dr. Nóra Németh, Dr. Judit Tax, Dr. Tamás Tóth, Dr. Zsolt Visnyei. I also express my gratitude to the assistants of the Central Laboratory, who were so kind to volunteer to join the studies as controls.

I am grateful to Prof. Dr. Márk Kollai for the most valuable years that I have spent in his study group as a medical student, for his thorough instructions and advices on my scientific work, and for his helpful cooperation in this study. I am grateful to my colleagues from the Institute of Human Physiology and Clinical Experimental Research, Semmelweis University, for their kind and valuable cooperation: Dr. Tamás Horváth, Ibolya Kocsis, Dr. Péter Studinger. I also remain with great gratitude to Dr. Levente Littvay, for his very important work, his helpfulness and his advices on statistical analyses.

I am especially grateful to Prof. Dr. Margit Abonyi, Prof. Dr. János Fehér, Prof. Dr. Péter Kempler and Prof. Dr. Endre Ibrányi, for their most helpful attitude from the first step of my researches, for their advices and kind cooperation.

I am most grateful to Prof. Dr. Hubert Blum, for his most kind and helpful attitude and very important advices on writing, submitting and managing manuscripts. I also thank him for the unforgettable and very useful scholarship times in his department at Freiburg University.

I am very grateful to Prof. Dr. György Jermendy, for giving me all the support to complete my PhD studies beside the clinical work.

References

[1] Lavanchy D. The global burden of hepatitis C. Liver Int 2009; 29 (Suppl 1): 74-81.

[2] Szabó E, Lotz G, Páska C, Kiss A, Schaff Zs. Viral hepatitis: new data on hepatitis C infection. Pathol Oncol Res. 2003; 9(4): 215-21.

[3] JF Perz, GL Armstrong, LA Farrington, YJ Hutin, BP Bell. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide, J Hepatol 2006; 45, pp. 529–538

[4] Prince AM, Brotman B, Grady GF, Kuhns WJ, Hazzi C, Levine RW, Millian SJ. Long-incubation post-transfusion hepatitis without serological evidence of exposure to hepatitis-B virus. Lancet. 1974;2(7875):241-6.

[5] Kuo G, Choo QL, Alter HJ, Gitnick GL, Redeker AG, Purcell RH, Miyamura T, Dienstag JL, Alter MJ, Stevens CE. An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. Science. 1989 21; 244(4902):362-4.

[6] Pár A. Hepatitis C vírus (HCV) infekció - 20 év után [Hepatitis C virus infection - after 20 years.] Orv Hetil. 2010;151(12):485-93.

[7] Houghton M. Discovery of the hepatitis C virus. Liver Int. 2009 Jan;29 Suppl 1:82-8.

[8] Gábor K, Iván H, Barnabás L. Intranuclear virus-like particles in HBsAg-and IHxAg negative acute hepatitis (hepatitis C?) Orv Hetil. 1976;117(48):2909-11.

[9] Roberts EA, Yeung L. Maternal-infant transmission of hepatitis C virus infection, Hepatology 2002; 36 (5 suppl 1): S106–S113

[10] Terrault NA. Sexual activity as a risk factor for hepatitis C, Hepatology 2002; 36 (5 suppl 1): S99–S105.

[11] Busch MP, Glynn SA, Stramer SL, Strong DM, Caglioti S, Wright DJ, Pappalardo B, Kleinman SH; NHLBI-REDS NAT Study Group. A new strategy for estimating risks of transfusion-transmitted viral infections based on rates of detection of recently infected donors. Transfusion 2005; 45, 254–264

[12] Alter MJ. Epidemiology of hepatitis C virus infection. World J Gastroenterol. 2007;13, 2436–2441.

[13] Daniel D, Grytdal S, Wasley A. (2009) Surveillance for acute viral hepatitis – United States, 2007. MMWR Surveill Summ 58: 1-27.

[14] Aceijas C, Rhodes T. Global estimates of prevalence of HCV infection among injecting drug users. Int J Drug Policy. 2007;18(5):352-8.

[15] Thorpe LE, Ouellet LJ, Hershow R, Bailey SL, Williams IT, Williamson J, Monterroso ER, Garfein RS. Risk of hepatitis C virus infection among young adult injection drug users who share injection equipment. Am J Epidemiol. 2002; 155, 645–653.

[16] Simonsen L, Kane A, Lloyd J, Zaffran M, Kane M. Unsafe injections in the developing world and transmission of bloodborne pathogens: a review. Bull World Health Organ 1999; 77: 789–800.

[17] Frank C, Mohamed MK, Strickland GT, Lavanchy D, Arthur RR, Magder LS, El Khoby T, Abdel-Wahab Y, Aly Ohn ES, Anwar W, Sallam I. The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. Lancet 2000; 355: 887–91.

[18] Rooney G and Gilson RJ. Sexual transmission of hepatitis C virus infection, Sex Transm Infect. 1998; 74, 399–404.

[19] Jain S, Goharkhay N, Saade G, Hankins GD and Anderson GD. Hepatitis C in pregnancy, Am J Perinatol 24 (2007), pp. 251–256.

[20] McIntyre PG, Tosh K and McGuire W. Caesarean section versus vaginal delivery for preventing mother to infant hepatitis C virus transmission, Cochrane Database Syst Rev 4 (2006) CD005546

[21] Stoszek SK, Abdel-Hamid M, Narooz S, El Daly M, Saleh DA, Mikhail N, Kassem E, Hawash Y, El Kafrawy S, Said A, El Batanony M, Shebl FM, Sayed M, Sharaf S, Fix AD, Strickland GT. Prevalence of and risk factors for hepatitis C in rural pregnant Egyptian women. Trans R Soc Trop Med Hyg 2006; 100: 102–7.

[22] P Klenerman and A Kim, HCV-HIV coinfection: simple messages from a complex disease, PLoS Med 4 (2007), p. e240.

[23] Bica I, McGovern B, Dhar R, Stone D, McGowan K, Scheib R, Snydman DR.. Increasing mortality due to end-stage liver disease in patients with human immunodeficiency virus infection. Clin Infect Dis 32 (2001), pp. 492–497.

[24] World Health Organization. Hepatitis C - global prevalence (update). Weekly Epidemiological Record, 2002, 77:41-48.

[25] Alter MJ. Epidemiology of viral hepatitis and HIV co-infection. J Hepatol 2006; 44(1 suppl): S6–S9.

[26] Centers for Disease Control and Prevention (CDC). Recommendations for prevention and control hepatitis C virus (HCV) infection and HCV-related chronic disease. MMWR 1998; 47: 1-39.

[27] Miller RH, Purcell RH. 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 U S A. 1990 Mar;87(6):2057-61.

[28] Bukh J, Purcell RH, Miller RH. Sequence analysis of the 5' noncoding region of hepatitis C virus. Proc Natl Acad Sci U S A. 1992 Jun 1;89(11):4942-6.

[29] Honda M, Beard MR, Ping LH, Lemon SM. A phylogenetically conserved stem-loop structure at the 5' border of the internal ribosome entry site of hepatitis C virus is required for cap-independent viral translation. J Virol. 1999 Feb;73(2):1165-74.

[30] Friebe P, Bartenschlager R. Genetic analysis of sequences in the 3' nontranslated region of hepatitis C virus that are important for RNA replication. J Virol. 2002 Jun;76(11):5326-38.

[31] Fang JW, Chow V, Lau JY. Virology of hepatitis C virus. Clin Liver Dis. 1997 Nov;1(3):493-514,

[32] Ogata N, Alter HJ, Miller RH, Purcell RH. Nucleotide sequence and mutation rate of the H strain of hepatitis C virus. Proc Natl Acad Sci USA 1991; 88: 3392–96.

[33] Dubuisson J. Hepatitis C virus proteins. World J Gastroenterol. 2007 May 7;13(17):2406-15.

[34] Kuiken C, Simmonds P. Nomenclature and numbering of the hepatitis C virus. Methods Mol Biol. 2009;510:33-53.

[35] Mellor, J., Holmes, E. C., Jarvis, L. M., Yap, P. L., and Simmonds, P. (1995) Investigation of the pattern of hepatitis C virus sequence diversity in different geographical regions: implications for virus classification. The International HCV Collaborative Study Group. J. Gen. Virol. 76, 2493–2507.

[36] Tokita, H., Okamoto, H., Luengrojanakul, P., Vareesangthip, K., Chainuvati, T., Iizuka, H. (1995) Hepatitis C virus variants from Thailand classifiable into five novel

genotypes in the sixth (6b), seventh (7c, 7d) and ninth (9b, 9c) major genetic groups. J. Gen. Virol.76, 2329–2335.

[37] Marsh M., Helenius A., Pybus, O. G., Charleston, M. A., Gupta, S., Rambaut, A. (2001) The epidemic behavior of the hepatitis C virus. Science 292, 2323–2325.

[38] Simmonds P. The origin and evolution of hepatitis viruses in humans. J. Gen. Virol. 2001; 82, 693–712.

[39] Cochrane A, Searle B, Hardie A, Robertson R, Delahooke T, Cameron S. (2002) A genetic analysis of hepatitis C virus transmission between injection drug users. J. Infect. Dis. 186, 1212–1221.

[40] Gervain J, Simon Jr G, Simon J; Hungarian Viral Hepatitis Gr. Genotype distribution of hepatitis C virus in the Hungarian population with chronic viral hepatitis C. Eur J Gastroenterol Hepatol. 2003; 15: 449-50.

[41] Puoti M, Zonaro A, Ravaggi A, Marin MG, Castelnuovo F, Cariani E. Hepatitis C virus RNA and antibody response in the clinical course of acute hepatitis C virus infection. Hepatology. 1992 Oct;16(4):877-81.

[42] Workowski KA, Berman SM. Sexually Transmitted Disease Guidelines, 2006. MMWR Recomm Rep. 2006; 55: 1-94.

[43] Hino K, Sainokami S, Shimoda K, Niwa H, Iino S. Clinical course of acute hepatitis C and changes in HCV markers. Dig Dis Sci. 1994 Jan;39(1):19-27.

[44] Seeff LB. Natural history of chronic hepatitis C. Hepatology. 2002 Nov;36(5 Suppl 1):S35-46. Review.

[45] Seeff LB. The history of the "natural history" of hepatitis C (1968-2009). Liver Int. 2009 Jan;29 Suppl 1:89-99.

[46] Ryder SD, Irving WL, Jones DA, Neal KR, Underwood JC; Trent Hepatitis C Study Group. Progression of hepatic fibrosis in patients with hepatitis C: a prospective repeat liver biopsy study. Gut. 2004 Mar;53(3):451-5.

[47] Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. Lancet 1997; 349: 825–32.

[48] Thein H-H, Yo Q, Dore GJ, Krahn MD. Estimation of stage-specific fibrosis progression rates in chronic hepatitis C virus infection: a meta-analysis and meta-regression. Hepatology 2008; 48: 418–38.

[49] El-Serag HB. Hepatocellular carcinoma and hepatitis C in the United States. Hepatology. 2002 Nov;36(5 Suppl 1):S74-83. Review.

[50] Kim WR. The burden of hepatitis C in the United States. Hepatology. 2002 Nov;36(5 Suppl 1):S30-4. Review.

[51] Lok AS, Seeff LB, Morgan TR, di Bisceglie AM, Sterling RK, Curto TM, Everson GT, Lindsay KL, Lee WM, Bonkovsky HL, Dienstag JL, Ghany MG, Morishima C, Goodman ZD; HALT-C Trial Group. Incidence of hepatocellular carcinoma and associated risk factors in hepatitis C-related advanced liver disease. Gastroenterology. 2009 Jan; 136(1):138-48.

[52] Di Bisceglie AM. Natural history of hepatitis C: its impact on clinical management. Hepatology. 2000 Apr;31(4):1014-8.

[53] Casiraghi MA, De Paschale M, Romano L, Biffi R, Assi A, Binelli G, Zanetti AR. Long-term outcome (35 years) of hepatitis C after acquisition of infection through mini transfusions of blood given at birth. Hepatology 2004; 39: 90–6.

[54] Kenny-Walsh E for the Irish Hepatology Research Group. Clinical outcomes after hepatitis infection from contaminated anti-D immune globulin. N Eng J Med 1999; 340: 1228–33.

[55] Wiese M, Berr F, Lafrenz M, Porst H, Oesen U. Low frequency of cirrhosis in hepatitis C (genotype 1) single-source outbreak in Germany: a 20-year multicenter study. Hepatology 2000; 32: 91–6.

[56] Wiley TE, Brown J, Chan J. Hepatitis C virus infection in African Americans: its natural history and histological progression. Am J Gastroenterol 2002; 97: 700–6

[57] Sterling RK, Stravitz RT, Luketic VA, Sanyal AJ, Contos MJ, Mills AS, Shiffman ML. A comparison of the spectrum of chronic hepatitis C between Caucasians and African Americans. Clin Gastroenterol Hepatol 2004; 2: 469–73.

[58] Kuzushita N, Hayashi N, Moribe T, Katayama K, Kanto T, Nakatani S, Kaneshige T, Tatsumi T, Ito A, Mochizuki K, Sasaki Y, Kasahara A, Hori M.. Influence of HLA haplotypes on the clinical courses of individuals infected with hepatitis C virus. Hepatology 1998; 27: 240–4.

[59] Tillmann HL, Chen DF, Trautwein C, Kliem V, Grundey A, Berning-Haag A, Böker K, Kubicka S, Pastucha L, Stangel W, Manns MP. Low frequency of HLA-DRB1*11 in hepatitis C virus induced end stage liver disease. Gut 2001; 48: 714–8.

[60] Kunzler S, Baumann M, Schirmacher F, Dries V, Bayer E, Gerken G, Dienes HP, Lohse AW. Prediction of progressive liver fibrosis in hepatitis C virus infection by serum and tissue levels of transforming growth factor-β. J Virol 2001; 8: 430–7.

[61] Promrat K, McDermott DH, Gonzalez CM, Kleiner DE, Koziol DE, Lessie M, Merrell M, Soza A, Heller T, Ghany M, Park Y, Alter HJ, Hoofnagle JH, Murphy PM, Liang TJ. Associations of chemokine system polymorphisms with clinical outcomes and treatment responses of chronic hepatitis C. Gastroenterology 2003; 124: 352–60.

[62] Leandro G, Mangia A, Hui J, Fabris P, Rubbia-Brandt L, Colloredo G, Adinolfi LE, Asselah T, Jonsson JR, Smedile A, Terrault N, Pazienza V, Giordani MT, Giostra E, Sonzogni A, Ruggiero G, Marcellin P, Powell EE, George J, Negro F; HCV Meta-Analysis (on) Individual Patients' Data Study Group. Relationship between steatosis, inflammation, and fibrosis in chronic hepatitis C: a meta-analysis of individual patient data. Gastroenterology 2006; 130: 1636–42.

[63] Ratziu V, Munteanu M, Charlotte F, Bonyhay L, Poynard T; LIDO Study Group. Fibrogenic impact of high serum glucose in chronic hepatitis C. J Hepatol 2003; 39: 1049–55.

[64] Monto A, Alonzo J, Watson J, Grunfeld C, Wright TL. Steatosis in chronic hepatitis C: relative contributions of obesity, diabetes mellitus, and alcohol. Hepatology 2002; 36: 720–36.

[65] Hourigan LF, Macdonald GA, Purdie D, Whitehall VH, Shorthouse C, Clouston A, Powell EE. Fibrosis in chronic hepatitis C correlates significantly with body mass index and steatosis. Hepatology 1999; 29: 1215–9.

[66] Ortiz V, Berenguer M, Rayon JM, Carrasco D, Berenguer J. Contribution of obesity to hepatitis C-related fibrosis progression. Am J Gastroenterol 2002; 97: 2408–14.

[67] Puoti C, Castellacci R, Montagnese F. Hepatitis C virus carriers with persistently normal aminotransferases: healthy people or true patients? Dig Liver Dis 2000; 32: 634–43.

[68] Cividini A, Rebucci C, Silini E, Mondelli MU. Is the natural history of hepatitis C virus carriers with normal aminotransferase really benign? Gastroenterology 2001; 121: 1526–7.

[69] Graham CS, Baden LR, Yu E, Mrus JM, Carnie J, Heeren T, Koziel MJ. Influence of human immunodeficiency infection on the course of hepatitis C virus infection: a meta-analysis. Clin Infect Dis 2001; 33: 562–69.

[70] Pontisso P, Gerotto M, Benvegnu L, Chemello L, Alberti A.. Co-infection by hepatitis B virus and hepatitis C virus. Antiviral Ther 1998; 3: 137–42.

[71] Bonkovsky H, Banner BF, Rothman AL. Iron and chronic viral hepatitis. Hepatology 1997; 25:759–68.

[72] Hutchison SJ, Bird SM, Goldberg DJ. Influence of alcohol on the progression of hepatitis C virus infection: a meta-analysis. Hepatology 2005; 3: 1150–9.

[73] Monto A, Patel K, Bostrom A, Pianko S, Pockros P, McHutchison JG, Wright TL. Risks of a range of alcohol intake on hepatitis C-related fibrosis. Hepatology 2004; 39: 826–34.

[74] Pessione F, Ramond M-J, Njapoum C, Duchatelle V, Degott C, Erlinger S, Rueff B, Valla DC, Degos F. Cigarette smoking and hepatic lesions in patients with chronic hepatitis C. Hepatology 2001; 34: 121–5.

[75] Harris HE, Ramsay ME, Andrews N, Eldridge KP; HCV National Register Steering Group. Hepatitis C virus. Clinical course of hepatitis C virus during the first decade of infection: cohort study. BMJ. 2002 Feb 23;324(7335):450-3.

[76] Seeff LB, Hollinger FB, Alter HJ, Wright EC, Cain CM, Buskell ZJ, Ishak KG, Iber FL, Toro D, Samanta A, Koretz RL, Perrillo RP, Goodman ZD, Knodell RG, Gitnick G, Morgan TR, Schiff ER, Lasky S, Stevens C, Vlahcevic RZ, Weinshel E, Tanwandee T, Lin HJ, Barbosa L. Long-term mortality and morbidity of transfusion-associated non-A, non-B, and type C hepatitis: A National Heart, Lung, and Blood Institute collaborative study. Hepatology. 2001 Feb;33(2):455-63.

[77] Lotz G, Szalay F, Firneisz G, Abonyi M, Lengyel G, Telegdy L, Ibrányi E, Nemes B, Kury F, Schaff Z, Simon S. Localization of hepatitis C virus RNA on human red blood cells by RT in situ PCR technique. Scand J Gastroenterol 2002, 37, 578-584.

[78] Burlone ME, Budkowska A. Hepatitis C virus cell entry: role of lipoproteins and cellular receptors. J Gen Virol. 2009 May;90(Pt 5):1055-70.

[79] Petit JM, Minello A, Duvillard L. Cell surface expression of LDL receptor in chronic hepatitis C: correlation with viral load. Am J Physiol Endocrinol Metab. 2007 Jul;293(1):E416-20.

[80] André P, Komurian-Pradel F, Deforges S, Perret M, Berland JL, Sodoyer M, Pol S, Bréchot C, Paranhos-Baccalà G, Lotteau V. Characterization of low- and very-low-density hepatitis C virus RNA-containing particles. J Virol. 2002 Jul;76(14):6919-28.

[81] Su AI, Pezacki JP, Wodicka L, Brideau AD, Supekova L, Thimme R, Wieland S, Bukh J, Purcell RH, Schultz PG, Chisari FV. Genomic analysis of the host response to hepatitis C virus infection. Proc Natl Acad Sci U S A. 2002 Nov 26;99(24):15669-74.

[82] Sklan EH, Charuworn P, Pang PS, Glenn JS. Mechanisms of HCV survival in the host. Nat Rev Gastroenterol Hepatol. 2009 Apr;6(4):217-27.

[83] Neumann-Haefelin C, Blum HE, Chisari FV, Thimme R. T cell response in hepatitis C virus infection. J Clin Virol. 2005 Feb;32(2):75-85.

[84] Diamond MS. Evasion of innate and adaptive immunity by flaviviruses. Immunol Cell Biol. 2003 Jun;81(3):196-206.

[85] Orland JR, Wright TL, Cooper S. Acute hepatitis C. Hepatology. 2001; 33(2):321-7.

[86] Pawlotsky JM. Use and interpretation of virological tests for hepatitis C. Hepatology. 2002;36 (5 Suppl 1):S65-73.

[87] Kau A, Vermehren J, Sarrazin C. Treatment predictors of a sustained virologic response in hepatitis B and C. J Hepatol. 2008; 49(4):634-51.

[88] Ghany MG, Strader DB, Thomas DL, Seeff LB; American Association for the Study of Liver Diseases. Diagnosis, management, and treatment of hepatitis C: an update. Hepatology. 2009; 49(4):1335-74.

[89] Kobayashi S, Takeda T, Enomoto M, Tamori A, Kawada N, Habu D. Development of hepatocellular carcinoma in patients with chronic hepatitis C who had a sustained virological response to interferon therapy: a multicenter, retrospective cohort study of 1124 patients. Liver Int 2007; 27: 186-191.

[90] Jacobson IM, Brown RS Jr, Freilich B, Afdhal N, Kwo PY, Santoro J. Peginterferon alfa-2b and weight-based or flat-dose ribavirin in chronic hepatitis C patients: a randomized trial. Hepatology 2007; 46: 971-981.

[91] Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL, Jr. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002; 347: 975-982.

[92] Khuroo MS, Khuroo MS, Dahab ST. Meta-analysis: a randomized trial of peginterferon plus ribavirin for the initial treatment of chronic hepatitis C genotype 4. Aliment Pharmacol Ther 2004; 20: 931-938.

[93] Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001; 358: 958-965.

[94] Hadziyannis SJ, Sette H Jr, Morgan TR, Balan V, Diago M, Marcellin P. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a

randomized study of treatment duration and ribavirin dose. Ann Intern Med 2004; 140: 346-355.

[95] Galossi A, Guarisco R, Bellis L, Puoti C. Extrahepatic manifestations of chronic HCV infection. J Gastrointestin Liver Dis 2007; 16: 65–73.

[96] Ferri C, Monti, M, La Civita L, Longombardo G, Greco F, Pasero G, Gentilini P, Bombardieri S, Zignego AL. Infection on peripheral blood momonuclear cells by hepatitis C virus in mixed cryoglobulinemia. Blood 1993; 82: 3701 - 3704.

[97] Agnello V, De Rosa FG. Extrahepatic disease manifestations of HCV infection: some current issues. J Hepatol 2004; 40: 341-352.

[98] Brouet JC, Clauvel JP, Danon F, Klein M, Seligmann M. Biologic and clinical significance of cryoglobulins. A report of 86 cases. Am J Med 1974; 57: 775 - 788.

[99] Pawlotsky JM, Roudot-Thoraval F, Simmonds P, Mellor J, Ben Yahia MB, André **C**, Voisin MC, Intrator L, Zafrani ES, Duval J, Dhumeaux D. Extrahepatic immunologic manifestations in chronic hepatitis C and hepatitis C virus serotypes. Ann Intern Med 1995; 122: 169–73.

[100] Saadoun D, Asselah T, Resche-Rigon M, Charlotte F, Bedossa P, Valla D, Piette JC, Marcellin P, Cacoub P. Cryoglobulinemia is associated with steatosis and fibrosis in chronic hepatitis C. Hepatology 2006; 43: 1337–45.

[101] Khella SL, Souayah N. Hepatitis C: a review of its neurologic complications. The Neurologist. 2002;8:101–106.

[102] Acharya JN, Pacheco VH. Neurologic complications of hepatitis C. Neurologist. 2008 May; 14(3):151-6.

[103] Nemni R, Sanvito L, Quattrini A, Santuccio G, Camerlingo M, Canal N. Peripheral neuropathy in hepatitis C virus infection with and without cryoglobulinaemia. J Neurol Neurosurg Psychiatry. 2003;74:1267–1271.

[104] Kanda T. Chronic hepatitis C infection and peripheral neuropathy; is mixed cryoglobulinemia really important? Intern Med. 2003;42:377–378.

[105] Maisonobe T, Leger JM, Musset L. Neurological manifestations in cryoglobulinemia. Rev Neurol (Paris). 2002;158:920 –924.

[106] Forton DM, Allsop JM, Main J, Foster GR, Thomas HC, Taylor-Robinson SD. Evidence for a cerebral effect of the hepatitis C virus. Lancet. 2001;358:38 –39.

[107] Morsica G, Bernardi MT, Novati R, Uberti Foppa **C**, Castagna A, Lazzarin A. Detection of hepatitis C genomic sequences in the cerebrospinal fluid of HIV-infected patients. J Med Virol. 1997; 53: 252–254.

[108]. Duncan G, Johnson RH, Lambie DG, Whiteside EA. Evidence of vagal neuropathy in chronic alcoholics. Lancet 1980; 2: 1053–7.

[109]. Kempler P, Váradi A, Szalay F. Autonomic neuropathy in liver disease. Lancet, 1989; 2(8675): 1332.

[110]. Oliver MI, Miralles R, Rubíes-Prat J, Navarro X, Espadaler JM, Solá R, Andreu M. Autonomic dysfunction in patients with non-alcoholic chronic liver disease. J Hepatol 1997; 26: 242–8.

[111]. Keresztes K, Istenes I, Folhoffer A, Lakatos PL, Horvath A, Csak T, Varga P, Kempler P, Szalay F. Autonomic and sensory nerve dysfunction in primary biliary cirrhosis. World J Gastroenterol 2004; 10: 3039–43.

[112]. Hendrickse MT, Thuluvath PJ, Triger DR. Natural history of autonomic neuropathy in chronic liver disease. Lancet 1992; 339(8807): 1462–4.

[113] Fleckenstein JF, Frank S, Thuluvath PJ. Presence of autonomic neuropathy is a poor prognostic indicator in patients with advanced liver disease. Hepatology 1996; 23: 471-475

[114] Valensi P. Diabetic autonomic neuropathy: what are the risks? Diabetes Metab 1998; 24(Suppl 3): 66-72

[115] Lazzeri C, La Villa G, Laffi G, Vecchiarino S, Gambilonghi F, Gentilini P, Franchi F. Autonomic regulation of heart rate and QT interval in nonalcoholic cirrhosis with ascites. Digestion 1997; 58: 580-586

[116] Moller S, Winberg N, Henriksen JH. Noninvasive 24-hour ambulatory arterial blood pressure monitoring in cirrhosis. Hepatology 1995; 22: 88-95

[117] Kempler P, Marton A, Tóth T, Keresztes K, Kádár É, Szalay F. May autonomic neuropathy play a role in the development of hyperdynamic circulation and portal hypertension in chronic liver diseases? (Hypotesis) Z. Gastroenterol 1996/b; 34: 316.

[118] Dreifus LS, Agarwal JB, Botvtnick EH (American College of Cardiology Cardiovascular Technology Assessment Committee). Heart rate variability for risk stratification of life-threatening arrhythmias. J Am Coll Cardiol 1993, 22. 948-50.

[119]. Pinna GD, Maestri R, Torunski A, Danilowicz-Szymanowicz L, Szwoch M, La Rovere MT, Raczak G. Heart rate variability measures: a fresh look at reliability. Clin Sci (Lond) 2007; 113: 131–40.

[121]. O'Brien IA, O'Hare P, Corrall RJM. Heart rate variability in healthy subjects: effect of age and the derivation of normal ranges for tests of autonomic function. Br Heart J. 1986; 55: 348-54.

[121]. Kardos A, Watterich G, de Menezes R, Csanády M, Casadei B, Rudas L. Determinants of spontaneous baroreflex sensitivity in a healthy working population. Hypertension 2001; 37(3): 911-6.

[122]. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Circulation 1996; 93: 1043–65.

[123]. Tank J, Baevski RM, Fender A, Baevski AR, Graves KF, Ploewka K, Weck M. Reference values of indices of spontaneous baroreceptor reflex sensitivity. Am J Hypertens 2000; 13: 268–75.

[124]. Mortara A, La Rovere MT, Pinna GD, Prpa A, Maestri R, Febo O, Pozzoli M, Opasich C, Tavazzi L. Arterial baroreflex modulation of heart rate in chronic heart failure: clinical and hemodynamic correlates and prognostic implications. Circulation 1997; 96: 3450–8.

[125]. Tsuji H, Larson MG, Venditti FJ. Impact of reduced heart rate variability on risk for cardiac events. The Framingham Heart Study. Circulation 1996; 94: 2850–5.

[126]. Ates F, Topal E, Kosar F, Karincaoglu M, Yildirim B, Aksoy Y, Aladag M, Harputluoglu MM, Demirel U, Alan H, Hilmioglu F. The relationship of heart rate variability with severity and prognosis of cirrhosis. Dig Dis Sci 2006; 51: 1614–8.

[127]. Fleisher LA, Fleckenstein JF, Frank SM, Thuluvath PJ. Heart rate variability as a predictor of autonomic dysfunction in patients awaiting liver transplantation. Dig Dis Sci 2000; 45: 340–4.

[128]. Foster GR, Goldin RD, Thomas HC. Chronic hepatitis C virus infection causes a significant reduction in quality of life in the absence of cirrhosis. Hepatology 1998; 27: 209–12.

[129]. Hilsabeck RC, Perry W, Hassanein TI. Neuropsychological impairment in patients with chronic hepatitis C. Hepatology 2002; 35: 440–6.

[130]. Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. Hepatology 1994; 19: 1513–20.

[131]. Spengler U, Nattermann J. Immunopathogenesis in hepatitis C virus cirrhosis. Clin Sci (Lond) 2007; 112: 141–55.

[132]. Brass V, Moradpour D, Blum HE. Hepatitis C virus infection: in vivo and in vitro models. J Viral Hepat 2007; 14(S1): 64–7.

[133]. Nemni R, Corbo M, Fazio R, Quattrini A, Comi G, Canal N. Cryoglobulinaemic neuropathy. A clinical, morphological and immunocytochemical study of 8 cases. Brain 1998; 111: 41–52.

[134]. Nemni R, Sanvito L, Quattrini A, Santuccio G, Camerlingo M, Canal N. Peripheral neuropathy in hepatitis C virus infection with and without cryoglobulinaemia. J Neurol Neurosurg Psychiatry 2003; 74: 1267–71.

[135]. Cacoub P, Poynard T, Ghillani P. Extrahepatic manifestations of chronic hepatitis C. MULTIVIRC Group. Multidepartment Virus C. Arthritis Rheum 1999; 42: 2204–12.

[136]. Ferri C, La Civita L, Cirafisi C, Siciliano G, Longombardo G, Bombardieri S, Rossi B. Peripheral neuropathy in mixed cryoglobulinemia: clinical and electrophysiologic investigations. J Rheumatol 1992; 19: 889–95.

[137]. Ammendola A, Sampaolo S, Migliaresi S, Ambrosone L, Ammendola E, Ciccone G, Di Iorio G. Autonomic neuropathy in mixed cryoglobulinemia.
J Neurol 2007; 254: 215–9.

[138]. Schmidt WN, Stapleton JT, LaBrecque DR. Hepatitis C virus (HCV) infection and cryoglobulinemia: analysis of whole blood and plasma HCV-RNA concentrations and correlation with liver histology. Hepatology 2000; 31: 737–44.

[139]. Nemni R, Sanvito L, Quattrini A, Santuccio G, Camerlingo M, Canal N. Peripheral neuropathy in hepatitis C virus infection with and without cryoglobulinaemia. J Neurol Neurosurg Psychiatry 2003; 74: 1267–71.

[140]. Agnello V, Abel G. Localization of hepatitis C virus in cutaneous vasculitic lesions in patients with type II cryoglobulinemia. Arthritis Rheum 1997; 40: 2007–15.

[141]. Ueno Y, Kondo K, Kidokoro N, Kobashi R, Kanaji K, Matsumura T. Hepatitis C infection and polymyositis. Lancet 1995; 346(8970): 319–20.

[142]. Authier FJ, Bassez G, Payan C , Guillevin L, Pawlotsky JM, Degos JD, Gherardi RK, Belec L. Detection of genomic viral RNA in nerve and muscle of patients with HCV neuropathy. Neurology 2003; 60: 808–12.

[143] Santoro L, Manganelli F, Briani C, Giannini F, Benedetti L, Vitelli E, Mazzeo A, Beghi E; HCV Peripheral Nerve Study Group. Prevalence and characteristics of

peripheral neuropathy in hepatitis C virus population. J Neurol Neurosurg Psychiatry 2006; 7: 626–9.

[144] Zekry A, McHutchison JG, Diehl AM. Insulin resistance and steatosis in hepatitis C virus infection. Gut 2005; 54: 903–6.

[145]Tomlinson DR, Gardiner NJ. Glucose neurotoxicity. Nat Rev Neurosci 2008;9:36-45.

[146]. Cacoub P, Ratziu V, Myers RP, Ghillani P, Piette JC, Moussalli J, Poynard T; Multivirc Group. Impact of treatment on extra hepatic manifestations in patients with chronic hepatitis C. J Hepatol 2002; 36: 812-8.

[147]. Briani C, Chemello L, Zara G, Ermani M, Bernardinello E, Ruggero S, Toffanin E, Gatta A, Battistin L, Cavalletto L. Peripheral neurotoxicity of pegylated interferon alpha: a prospective study in patients with HCV. Neurology 2006; 67: 781–85

[148]. Gastineau DA, Habermann TM, Hermann RC. Severe neuropathy associated with low-dose recombinant interferon-alpha. Am J Med 1989; 87: 116.

[149]. Fried MW. Side effects of therapy of hepatitis C and their management. Hepatology 2002; 36(Suppl. 1): S237–44.

[150]. Reichard O, Andersson J, Schvarcz R, Weiland O. Ribavirin treatment for chronic hepatitis C. Lancet 1991; 337: 1058-61.

[151]. Di Bisceglie AM, Shindo M, Fong TL, Fried MW, Swain MG, Bergasa NV, Axiotis CA, Waggoner JG, Park Y, Hoofnagle JH. A pilot study of ribavirin therapy for chronic hepatitis C. Hepatology. 1992; 16: 649-54.

[152]. Poynard T, Marcellin P, Lee SS. Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. International Hepatitis Interventional Therapy Group (IHIT). Lancet 1998; 352: 1426-32.

[153]. McHutchison JG, Gordon SC, Schiff ER, Shiffman ML, Lee WM, Rustgi VK, Goodman ZD, Ling MH, Cort S, Albrecht JK.. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. N Engl J Med 1998; 339: 1485-92.

[154]. Beuthien W, Mellinghoff HU, Kempis J. Vasculitic complications of interferon-alpha treatment for chronic hepatitis C virus infection: case report and review of the literature. Clin Rheumatol 2005; 24: 507-15.

[155]. Gupta R, Singh S, Tang R, Blackwell TA, Schiffman JS. Anterior ischemic optic neuropathy caused by interferon alpha therapy. Am J Med 2002; 112: 683–84.

[156] Daniel AL, Houlihan JL, Blum JS, Walsh JP. Type B insulin resistance developing during interferon-alpha therapy. Endocr Pract. 2009;15(2):153-7.

[157] Becker K, Görlach I, Frieling T, Häussinger D. Characterization and natural course of cardiac autonomic nervous dysfunction in HIV-infected patients. AIDS 1997; 11: 751-7.

[158] Sakhuja A, Goyal A, Jaryal AK, Wig N, Vajpayee M, Kumar A, Deepak KK. Heart rate variability and autonomic function tests in HIV positive individuals in India. Clin Auton Res 2007; 17: 193-6.

[159] Sanya EO, Tutaj M, Brown CM. Abnormal heart rate and blood pressure responses to baroreflex stimulation in multiple sclerosis patients. Clin Auton Res 2005; 15: 213-8.

[160] Flachenecker P, Reiners K, Krauser M. Autonomic dysfunction in multiple sclerosis is related to disease activity and progression of disability. Mult Scler 2001; 7: 327-34.

[161] Besedovsky HO, del Rey A. Immune-neuro-endocrine interactions: facts and hypotheses. Endocr Rev 1996; 17: 64-102.

[162] Elenkov IJ, Wilder RL, Chrousos GP, Vizi ES. The sympathetic nerve - an integrative interface between two supersystems: the brain and the immune system. Pharmacol Rev 2000; 52: 595-638.

[163] Biddle C. The neurobiology of the human febrile response. AANA J 2006; 74: 145-50.

[164] Katafuchi T, Kondo T, Take S, Yoshimura M. Brain cytokines and the 5-HT system during poly I:C-induced fatigue. Ann N Y Acad Sci 2006; 1088: 230-7.

List of publications

List of publications regarding to dissertation

- **Osztovits J,** Horváth E, Tax J, Csihi L, Horváth T, Littvay L, Tóth T, Abonyi M, Lakatos PL, Kollai M, Fehér J, Szalay F, Blum HE:
  Reversible autonomic dysfunction during antiviral treatment in patients with chronic hepatitis C virus infection.
  Hepat Mon 2011; 11(2): 114-8. **IF: 0.793**

- **Osztovits J,** Horváth T, Abonyi M, Tóth T, Bekő G, Csák T, Lakatos PL, Fehér J, Littvay L, Kempler P, Kollai M, Szalay F:
  Chronic hepatitis C virus infection associated with autonomic dysfunction
  Liver Int 2009; 29: 1473-8. **IF: 3.840**

List of further publications

- **Osztovits J**, Horváth T, Littvay L, Steinbach R, Jermendy A, Tárnoki A, Tárnoki D, Métneki J, Kollai M, Jermendy G.:
  Effects of genetic vs. environmental factors on cardiovascular autonomic function: a twin study.
  Diabet Med. 2011 Jun 16 **IF: 3.036**

- Jermendy G, Littvay L, Steinbach R, Jermendy A, Tárnoki A, Tárnoki D, Métneki J, **Osztovits J**:
  Heritability of the risk factors characteristic for the metabolic syndrome: a twin study.
  Orv Hetil. 2011; 152(32):1265-71. Hungarian.

- Papp M, Norman GL, Vitalis Z, Tornai I, Altorjay I, Foldi I, Udvardy M, Shums Z, Dinya T, Orosz P, Lombay B Jr, Par G, Par A, Veres G, Csak T, **Osztovits J,** Szalay F, Lakatos PL. Presence of anti-microbial antibodies in liver cirrhosis--a tell-tale sign of compromised immunity?
  PLoS One. 2010 Sep 23;5(9):e12957. **IF: 4.351**

- Altorjay I, Vitalis Z, Tornai I, Palatka K, Kacska S, Farkas G, Udvardy M, Harsfalvi J, Dinya T, Orosz P, Lombay B Jr, Par G, Par A, Csak T, **Osztovits J,** Szalay F, Csepregi A, Lakatos PL, Papp M. Mannose-binding lectin deficiency confers risk for bacterial infections in a large Hungarian cohort of patients with liver cirrhosis.
  J Hepatol. 2010 Sep;53(3):484-91. **IF: 7.818**

- Beko G, Hagymasi K, Szentmihalyi K, Banyai E, **Osztovits J,** Fodor J, Feher J, Blazovics A.
  Sex-dependent alterations in erythrocyte trace element levels and antioxidant status after a month of moderate daily red wine consumption.
  Eur J Gastroenterol Hepatol. 2010 Feb;22(2):185-91. **IF: 1.662**

- **Osztovits J,** Balázs C, Fehér J.
  H1N1-influenza pandemic, 2009
  Orv Hetil. 2009; 150(50):2265-73. Hungarian.

- Fischer S, Lakatos PL; Hungarian IBD Study Group, Lakatos L, Kovacs A, Molnar T, Altorjay I, Papp M, Szilvasi A, Tulassay Z, **Osztovits J,** Papp J, Demeter P, Schwab R, Tordai A, Andrikovics H:
  ATP-binding cassette transporter ABCG2 (BCRP) and ABCB1 (MDR1) variants are not associated with disease susceptibility, disease phenotype response to medical therapy or need for surgeryin Hungarian patients with inflammatory bowel diseases.
  Scand J Gastroenterol. 2007 Jun;42(6):726-33. **IF: 1.790**

- Lakatos PL, Hitre E, Szalay F, Zinober K, Fuszek P, Lakatos L, Fischer S, **Osztovits J,** Gemela O, Veres G, Papp J, Ferenci P.:
  Common NOD2/CARD15 variants are not associated with susceptibility or the clinicopathologic characteristics of sporadic colorectal cancer in Hungarian patients.
  BMC Cancer. 2007 Mar 27;7:54. **IF: 1.869**

- Folfoffer A, Ferenci P, Csak T, Horvath A, Hegedus D, Firneisz G, **Osztovits J,** Kosa JP, Willheim-Polli C, Szonyi L, Abonyi M, Lakatos PL, Szalay F:
  Novel mutations of ATP7B gene among 109 Hungarian patients with Wilson disease.
  Eur J Gastroenterol Hepatol. 2007 Feb;19(2):105-11. **IF: 1.890**

- Csák T, Folhoffer A, Horváth A, **Osztovits J,** Papp J, Görög D, Kóbori L, Szalay F:
  Xanthomatosis and extreme hypercholesterolemia after laparoscopic cholecystectomy. Total reversibility following surgical treatment of iatrogenous stenosis of the common bile duct
  Orv Hetil. 2006 Apr 16;147(15):705-10. Hungarian.

- Csák T, Folhoffer A, Horváth A, **Osztovits J,** Halász J, Diczházi Cs, Schaff Zs, Szalay F:
  Autoimmun hepatitis, coeliakia and Holmes-Adie syndroma. A case report.
  Magy Belorv Arch 2006; 59: 55-58. Hungarian.

- Urbancsek J, Hauzman E, Lagarde AR, **Osztovits J,** Papp Z, Strowitzki T.
  Serum CA-125 levels in the second week after embryo transfer predict clinical pregnancy.
  Fertil Steril. 2005 May;83(5):1414-21 **IF: 3.970**