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REVIEW ARTICLE

Hepatitis C: an Overview of Various Laboratory Assays with their Mode of Diagnostic Cooperation

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SUMMARY

Background: **This review describes in chronological order the different assays for hepatitis C virus (HCV) antibodies, for the core antigen and for the HCV-RNA.**
Methods: **By ascending chronological order, the enzyme-linked immunosorbent assay (ELISA), rapid diagnostic tests (RDTs), HCV-Ab IgG avidity index (HCV AI), and Cy3-labeled microarray assay have been described for HCV antibodies in addition to ELISA for the total HCV core antigen (Ag).**
Results: **The recombinant immunoblot assay (RIBA) is a confirmatory test for HCV-Ab in blood, which is no longer needed due to the use of the sensitive third and fourth generation ELISA in addition to HCV-RNA detection by the Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR). ELISA and RIBA measure current and prior exposure to HCV infection, but cannot discriminate between the two. RT-PCR is performed after ELISA for the diagnosis of HCV infection whether acute, chronic, false positive or false negative ELISA.**
Conclusions: **The cooperation between ELISA and RT-PCR in the diagnosis of HCV infection has been tabulated and discussed. HCV genotyping and subtyping testing is essential in pre-treatment evaluation of the patients for setting valuable treatment strategies and in understanding the epidemiology of the virus.**
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INTRODUCTION

Hepatitis C virus (HCV) is an RNA virus belonging to genus Hepacivirus within the family Flaviviridae. It is estimated that about 3% of the world population is infected, with chronicity of about 80%, and is primarily parentally transmitted via blood transfusion, injection drug use, unsafe injection practices, and other health care related procedures [1].
The laboratory diagnostic tools of HCV infection are of two categories: indirect tests (serological assays) detecting specific antibody to HCV (anti-HCV) and direct assays that are directed toward the components of HCV viral particles, such as HCV RNA and core antigen. Both direct and indirect virological tests cooperate in the diagnosis of infection, therapeutic decision-making,

and assessment of virological response to therapy. In this review, various laboratory assays will be gathered and discussed in a chronological manner to show their concepts, developments, and principles and to demonstrate their applications and cooperation in the diagnosis of HCV infection.

I-Tests for HCV antibodies (HCV-Ab)

Enzyme-linked immunosorbent assays (ELISA)

Late in the last century, Hepatitis C Virus (HCV), was discovered as the etiologic agent for non-A non-B hepatitis [2-4], and testing began with the indirect enzyme-linked immunosorbent assays (indirect ELISA or indirect EIA) for detection of HCV-antibodies (HCV-Ab) appeared world-wide as 1st generation, then 2nd, 3rd, and 4th generation, with improvement in sensitivity and specificity [5-7]. In the indirect ELISA, the tested HCV-Abs interact with the recombinant HCV-proteins immobilized altogether on a plastic well, followed by washing, incubation in conjugate (peroxidase-labeled goat anti-human Ig), washing, and incubation with a substrate to yield a color. The intensity of the color is proportional to the concentration of the HCV-Abs. The RNA genome of HCV encodes at least nine proteins including six nonstructural (NS) proteins (NS-2, -3, -4A, -4B, -5A, and -5B) and three structural proteins (El, E2, and Core) [5-7]. The original, 1st generation HCV-Ab EIA utilized a recombinant protein, clOO-3, derived from the non-structural (NS-4) region of the viral genome as the solid-phase (immobilized) antigen [8]. The test was widely used, but found to possess low sensitivities for a high-prevalence population (approximately 80%) [9], inability to discover all HCV patients [10], long "window-phase" [11], and a high rate of false-positive reaction such as in primary biliary cirrhosis [12], rheumatoid arthritis [13], malaria [14], paraproteinemia [15], and in chronic active hepatitis [16], chronic liver disease [17] and autoimmune hepatitis [18].

Second generation ELISA (or EIA) included recombinant HCV antigens: c33c from the nonstructural NS-3 region, and structural (c22-3) protein from the core region of HCV [19,20]. They detected HCV-Ab at much higher rates [21]. The mean window-phase was reduced from 16 weeks (with first generation EIAs) to 10 weeks and the sensitivities were increased to about 95% [9]. In 1996, the U.S Food and Drug Administration (FDA) approved a third generation EIA that added a fourth antigen (NS-5) which allowed earlier detection of antibodies (26 days earlier) [22] and increased the sensitivity up to 97% [6]. Therefore, third generation EIA (and ELISA) for HCV-Ab was considered to be more sensitive and specific than those of the second generation [23,24].

Sensitive 4th generation ELISA has also been used, with increased sensitivity for NS (-3, -4A, -4B, -5A) in addition to the Core region, for which the antigens (NS-3 and NS-4) were derived from genotypes 1a, 1b, 2, and 3 [25]. The INNOTEST HCV-Ab IV showed a specificity of 99.8% and enhanced sensitivity [7].

ELISAs require sophisticated equipment, regular maintenance, a constant electricity supply, and skilled technicians. Thus, they are suitable for testing large numbers of samples per day, in blood banks or for surveillance studies such as screening of baby boomers. The generation born between 1945 and 1965 (baby boomers) account for approximately 3/4 of all chronic HCV infections in the United States. Thus, their screening for HCV infection was recommended by the Centers for Disease Control and Prevention (CDC) [26] and by the US Preventive Services Task Force [27-29].

Rapid diagnostic tests (RDTs)

As described above, ELISAs are sensitive and specific but sophisticated. Thus, they are called laboratory tests as they are not suitable for preliminary or emergency medical screening. Alternatively, Simple/Rapid tests are better for emergency testing, and in smaller laboratories with low numbers of tests per day. These are the tests that are quick (results in minutes) and easy to perform. They also allow point-of-care testing in primary care for different things that formerly only a laboratory test could measure [30,31]. They include agglutination, immuno-dot, immuno-chromatographic and/or immuno-filtration techniques. In 2010, the FDA approved a Rapid Antibody Test (OraQuick HCV), in which HCV antigens (HCV-Ag) are fixed as a test strip on a cellulose membrane. HCV antibodies (if present) bind to HCV-Ag and then to colloidal gold particles (stored in the area) forming a red line indicating positivity. The test can be used for persons at risk for hepatitis or for those with signs or symptoms of hepatitis. The test strip can be used with a sample collected from a finger stick, venipuncture whole blood or oral fluids [32]. Screening for HCV in the emergency department (ED) has been found to be feasible, albeit costly [33,34].

HCV-Ab IgG Avidity Index (HCV AI) for HCV-Ab

HCV-Ab IgG Avidity Index (HCV AI) can be determined by ELISA by testing two aliquots of 200 mL (for each serum sample), one diluted 1:10 with 1 M guanidine and the other with phosphate-buffered saline (PBS) and incubated for 10 minutes at room temperature. HCV AI is the ratio between the OD at 492 nm from the guanidine wells and the OD from the PBS wells [35]. HCV AI, within the first 8 days following the onset of clinical symptoms, may be useful in identifying actual acute HCV infection cases [36].

The recombinant immunoblot assay (RIBA) as complementary for HCV-Ab

Initially, the main problem encountered with HCV testing was the lack of confirmatory tests. Recombinant immunoblot assays (RIBA) [13,37,38] had been developed as supplemental tests to discriminate between true- and false-positive results for samples that are repeatedly reactive by ELISAs [39]. RIBA is similar, in principle, to ELISA except that the recombinant and synthetic HCV-proteins are immobilized as bands on a nitrocellulose

strip rather than a plastic well in addition to a washing step after the final incubation with substrate to yield colored bands. Second generation RIBA (RIBA HCV 2.0) consisted of a nitrocellulose strip solid support on which four bands of recombinant HCV proteins are immobilized. The four bands contained four different antigens: the 5-1-1 antigen, the c100-3 recombinant antigen, the c33-c recombinant antigen, and the c22-3 antigen (respectively derived from the NS-4 region, the NS-4 region, the NS-3 region, and from the core region of the HCV genome). Since 1992, a greater specific and sensitive third-generation RIBA HCV strip immunoblot system (RIBA HCV 3.0 SIA) has been widely used, which had four significant differences from RIBA HCV 2.0: 1) it included a recombinant antigen from the NS-5 region, 2) the recombinant c22-3 antigen was replaced by a four-epitope core synthetic peptide c22(p), 3) the 5-1-1 and c100-3 recombinant antigens were replaced by a mixture of synthetic peptides c100 (p), and 4) more c33-c recombinant antigen was used [40-44]. However, 3rd generation ELISA tests for HCV-Ab are both highly sensitive and specific to the extent that only one single ELISA determination is necessary for the diagnosis of HCV infection, without the need for confirmation by RIBA. PCR for HCV-RNA detection resolves weakly positive or negative ELISA results in cases that are clinically compatible with hepatitis C [45].

Fluorescent cyanin3 (Cy3)-labeled microarray assay for HCV-Ab detection

In 2008, a new inexpensive, specific, and sensitive method, for the massive sample diagnosis, was described, in which the target HCV-proteins are immobilized altogether in gel in each well of a new 96-well chip-format plate; then the bound HCV-Abs are detected by cy3-labeled anti-human Ig [46]. The sol-gel contents included tetraethoxysilane, PEG8000, HCl, and sodium phosphate (pH 7.5) [47]. Each individual well was spotted with four different HCV antigens (Core, NS-5, NS-3, and E1/E2) that conferred 1000 times more sensitivity than that of the ELISA with high specificity (98.78%) yielding significantly fewer false-positive results than did the ELISA [46].

II-Tests for components of HCV particles (core antigen and RNA)

Tests for HCV core antigen detection

The availability of an anti-core antigen (Ag) monoclonal antibody allowed development of an ELISA detecting and quantifying total HCV core Ag in serum and plasma of HCV-infected patients, which required an initial step of immune complex dissociation to remove bound anti-core antibodies prior to the antigen detection with the monoclonal antibodies [48].

It was claimed to be accurate, precise, and specific but cannot be used for HCV RNA values below 20,000 IU/mL, making its clinical usefulness limited as HCV-RNA detection methods are more sensitive [48].

A new generation chemiluminescence immunoassay (CLIA) based quantitative test (Architect HCV Ag Test, Abbot, Germany) had been shown to possess a sensitivity comparable to that of end point PCR (~1,000 IU/mL) but less than that of real time PCR [49, 50].

The Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) for viral RNA

Polymerase Chain Reaction (PCR) [51-54] is a technology using molecular biology discovered in 1985 by Karry Mullis [53]. This method allows the amplification *in vitro* of a specific region of DNA many million-fold, in order to produce enough DNA to be adequately tested [53].

The Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) [55] is used for amplification of RNA (e.g., HCV-RNA) which has to be converted to cDNA by reverse transcription, using reverse transcriptase, and then PCR is performed [56,57]. RT-PCR is performed after extraction of the HCV-RNA from serum, which is carried out by using extraction kits (such as QIAGEN) according to the methods of Vogelstein and Gillespie (1979) [58] and Stull and Pisano (2001) [59].

Immunoserological tests (such as ELISA) measure current and/or prior exposure to HCV infection, but cannot discriminate between the two [60]; therefore, RT-PCR, which detects HCV-RNA, is performed for viremia detection within a few days after exposure to the virus and many weeks before elevation of transaminases and antibody titre [61-63]. This helps in monitoring anti-viral treatment for chronic HCV infection [64], which depends on the viral load [65], and in the diagnosis of vertical transmission of HCV from chronically-infected mothers to their offspring [66,67]. The cooperation between ELISA and RT-PCR in the diagnosis of HCV infection whether acute, chronic, false positive or false negative ELISA will be discussed later on, in Table 1. The PCR-amplified DNA can also be generated and identified by using the automated technology such as Cobas Amplicor RT-PCR qualitative assay with the limit of detection of less than 50 IU/mL and can be used to confirm viremia and assess ultimate treatment response [68] and SMART CYCLERII, a real-time PCR thermo-cycler, by which thermal and optical data can be monitored in real time, and graphs of temperature, growth, and melt curves are displayed during data collection [69].

HCV-RNA transcription-mediated amplification (HCV TMA) qualitative test

Based on transcription-mediated amplification (HCV TMA), a highly sensitive and reliable qualitative chemiluminescent assay for detection of HCV-RNA in human serum or plasma, was developed and manufactured [70, 71]. In HCV TMA, following a lysing step, target HCV-RNA is captured on magnetic particles coated with oligonucleotides complementary to the 5′ untranslated region of the HCV genome [72] in separate poly-

Table 1. Diagnosis of HCV infection according to HCV-Ab and HCV- RNA [1].

Test results		Case	Note
Ab [2]	RNA [3]		
- [4]	- [4]	False negative?	The first 1 - 3 weeks after accidental parenteral exposure
- [5]	- [5]	Unlikely acute hepatitis C	Patient whose liver disorders are due to another cause or "occult HCV infection"
+ [6]	- [6]	Unlikely acute hepatitis C	Healthy carriers, possible resolved infection, "occult HCV infection", transient patients or PCR inhibition
+ [7]	? [7]	Chronic hepatitis C	Detection of HCV-RNA confirms chronic HCV infection
+ [7]	+ [7]	Chronic hepatitis C	Chronicity is certain in a patient with chronic liver disease
- [4]	+ [4]	Chronic hepatitis C with false negative serology	Occasionally, immunocompromised, hemodialysis, agammaglobulinemic or mixed cryoglobulinemic patients have false- negative serology results
+ [8]	? [8]	False positive Ab	If absence of HCV-RNA on at least twice at 6 months apart
- [9]	+ [9]	Acute hepatitis C (window phase)	Will be confirmed by subsequent seroconversion
+ [10]	+ [10]	Acute hepatitis C	HCV IgG avidity index and serial measurements of the HCV-Ab IgM titres may help

propylene tubes, followed by incubation, washing, then amplification with an isothermal TMA process [70,71]. Detection of the amplified product is based on hybrid protection and the dual kinetic assays [72,73], using HCV probe reagent (chemiluminescent oligonucleotide probe) which produces a chemiluminescent signal that is read as relative light units (RLU) [70,71]. Qualitative HCV TMA has a sensitivity of 5.3 IU/mL [74] and is able to equally detect different HCV genotypes [61,75, 76]. The conversion of the qualitative HCV TMA assay to a quantitative assay has been described [77].

Genotyping of the extracted RNA

HCV genotyping was described by sequence-based PCR products analysis techniques, via genotype-specific antibodies [78], restriction endonuclease digestion [79], reverse hybridization against genotype-specific probes [80], direct sequencing [81,82], and annealing genotype-specific capture probes (AGSCP) [83]. Six major genotypes (1-6) have been characterized [84], with subtypes la to 1c, 2a to 2d, 3a to 3f, 4a to 4k, 5a, and 6a, and new (sub) types are continually being discovered [85-87]. Although in one study [88] 12 genotypes were predicted, but currently, the existence of six major HCV types is generally accepted. Debates are ongoing about whether the extra types are real distinct types or subtypes of types 3 and 6 [89].

Different methods for HCV-genotyping have been reported, including detailed sequence homology studies [82], restriction fragment length polymorphisms of amplified PCR products [83,90], PCR amplification with subtype-specific primers [91-95], and line probe assay (LiPA) based on RT-PCR followed by hybridization with specific probes (for each genotype) immobilized (as parallel lines) on nitrocellulose strips [96]. Genotyping and subtyping for HCV are clinically important in the understanding of HCV disease and therapy options [81,97-100].

III-Cooperation between HCV-Ab and HCV-RNA results in the diagnosis of HCV infection

Modes of cooperation between HCV-Ab and HCV-RNA results in the diagnosis of HCV infection can be classified into five categories: False negative (inquiry?), unlikely acute hepatitis C, chronic hepatitis C, False +veAb and acute hepatitis C (Table 1). The following discussion is a stepwise description of such cooperation according to the superscripts shown in Table 1.

Superscript 1

In the diagnosis of HCV infection; HCV-Ab, HCV-RNA, and serum liver chemistry values are commonly used, but the latter is not necessary from a virological point of view because up to 40% of viraemic patients can have a normal aminotransferase value on a single determination [101].

Only one single, third generation ELISA determination is necessary for the diagnosis of HCV infection without the need for confirmation of positive or weakly positive ELISAs with RIBA because HCV-RNA detection by RT-PCR helps to resolve weakly positive or negative ELISA results when clinically compatible with hepatitis C [45].

Cooperation between HCV-Ab and HCV-RNA results does not apply in case of offspring born to HCV-infected mothers, where the diagnosis depends on HCV-RNA detection, because maternal IgG is passively transferred *in utero* and remains detectable for several months after

delivery [102-104], while HCV-RNA can be detected within a few days after delivery and then persists or is cleared spontaneously [104]. Cord blood may get contaminated by maternal blood and should never be used for HCV-RNA testing in the diagnosis of prenatal HCV infection [105,106].
Routine testing of pregnant women for HCV-Ab is not recommended, because the number of pregnant women who would be positive is expected to be low, in addition to the lack of measures to prevent perinatal transmission of HCV, and no licensed therapy or guidelines for the treatment of HCV-infected infants or children exist [105-108].
Indeed, in patients who have no indication for therapy or have a contra-indication to the use of antiviral drugs, neither HCV-Ab nor the HCV-RNA are useful because these virological tests have no prognostic value as their loads do not correlate with the severity of liver inflammation or fibrosis, nor with their progression [109]. Alternatively, in untreated patients, the severity of liver inflammation and fibrosis must be evaluated every 3 - 5 years by other means such as liver biopsies or non-invasive serological or ultrasound-based testing [110].

Superscript 2
HCV-Ab should be tested by a sensitive and specific technique such as 3rd and 4th generation ELISA [6,7, 23,24], which mainly detect HCV-Ab IgG class, since HCV-Ab IgM class can be detected in 50 - 93% of patients with acute HCV infection and in 50 - 70% of chronic cases [111].

Superscript 3
HCV-RNA should be tested by a sensitive technique detecting ≤ 50 HCV-RNA IU/mL [112,113], such as qualitative Cobas Amplicor RT-PCR which detects < 50 IU/mL and can be used to confirm viremia [68,69], or by transcription-mediated amplification (HCV TMA) [70,72].
HCV-RNA detection (qualitative and quantification) helps to diagnose chronic HCV infection, identify patients who need antiviral therapy, and monitor the virological responses to antiviral therapy [108,114]. Qualitative HCV-RNA assays detect viral genomes confirming an active infection and demonstrate its presence 4 - 6 weeks before antibody seroconversion takes place [115].
Most qualitative HCV-RNA tests are 10- to 100-fold more sensitive than quantitative HCV-RNA tests, and up to 1,000-fold more sensitive than the HCV core antigen assay, making them the method of choice for confirming active infection and for assessing viral clearance in response to therapy [14,116].

Superscript 4
It was estimated that the rate of HCV seroconversion among health care workers after a needle stick injury (parenteral) exposure is 0 - 7% [106].
However, after an accidental parenteral exposure to the virus, negative HCV-RNA results should be retested a few weeks later, because HCV-RNA is detectable in serum within 1 - 3 weeks after accidental parenteral exposure to the virus and weeks before the onset of ALT elevations or the appearance of HCV-Ab [117,118]. Keeping in mind that occasionally immunocompromised patients, patients undergoing hemodialysis, and patients with agammaglobulinemia and with mixed cryoglobulinemia have false-negative serology results (detectable HCV replication in the absence of HCV-Abs), these patients may require HCV-RNA testing for diagnosis [119,122].

Superscript 5
Acute hepatitis C is very unlikely if both HCV-Abs and HCV-RNA are absent [109]; thus, patients with symptoms of acute hepatitis of uncertain origin that are negative HCV-Ab should undergo qualitative HCV-RNA testing [123]. However, negative HCV-Ab and negative serum HCV-RNA but positive liver HCV-RNA have been described in “occult HCV infection” in which the patients have abnormal liver function tests of unknown etiology since the patients lacks markers of HBV infection and lacks clinical or biochemical evidence of autoimmunity, genetic or metabolic disorders, alcohol intake or drug toxicity [124]. Most patients (70%) with intrahepatic HCV-RNA also have HCV-RNA in their peripheral blood mononuclear cells (PBMC) [124,125].

Superscript 6
Acute hepatitis C is also unlikely if HCV-Abs are present without HCV-RNA [109]. However, about 30% of HCV-Ab positive with HCV-RNA negative have been found to have significant liver disease on biopsy [126, 127]; thus, one should consider other possibilities for the negative HCV-RNA result such as “healthy” HCV-carriers, infection resolving, RNA transient drop, occult HCV-infection, and technical inhibition of PCR. Healthy HCV-carriers are those patients with positive HCV-Ab but negative for serum HCV-RNA and normal ALT levels, and considered to have encountered and cleared HCV at some time in the past [109].
It has been estimated that in 15 - 25% of HCV-infected patients, the infection resolves and the patients recover from hepatitis C [118,128].
A temporary (transient) drop in the level of viremia below the detection limit should be taken into consideration [118], which may occur due to partial control of viral replication (by the immune response) before replication escapes and chronic infection is established [129,130]. These patients should however be retested after a few weeks because HCV-RNA can be transient undetectable [129].
Patients, who are HCV-Ab positive with undetectable serum viral RNA and normal ALT levels, should be verified if they could have HCV-RNA in their liver and PBMC, which is another form of “Occult HCV infection” (see above, superscript 5) [125,131,132].

It is also important to rule out the possibility of technical inhibition of PCR due to the presence of heparin in the collected samples, which can be solved using an internal control incorporated in each test tube from the extraction step [133]. In addition to ruling out the possibility of losing a little of the RNA due to improper storing or as a result of repeated thawing and freezing [134] and in spite of other contrary results, the RNA level remained stable during repeated thawing and freezing for about 8 cycles [135].

Superscript 7
When an individual is found to be HCV-Ab positive during blood donation or screening of at-risk populations, then HCV-RNA should be tested since the PCR positive result, in such circumstances, will confirm chronic infection [119,136], keeping in mind that the persistence of HCV-RNA for more than 6 months defines chronic HCV infection [101], and that a continuing hepatitis disease without improvement for at least six months defines chronic hepatitis [137]. Chronicity is also certain when patients have clinical or biological signs of chronic liver disease [45,138].
Chronic infection is often not symptomatic [139] until evidence of liver failure becomes clinically apparent, in which the rate of progression to cirrhosis is usually slow, with 20 or more years elapsing between infection and the development of serious complications [140-142].

Superscript 8
If HCV-RNA is negative and HCV-Abs are positive, false positive EIA may occur, the exact prevalence of which is unknown and which cannot be differentiated from the pattern of patients who have recovered from a past HCV infection [109]. False positive EIA was seen in patients with autoimmune diseases [16,143], in neonates born to mothers with chronic HCV infection since passively transferred maternal HCV-Abs may be detected in the children of HCV-infected mothers for up to 1 year [106,144], a low viral load below the detection limit of PCR, inhibition of PCR due to the presence of heparin in the collected samples [133], and improper storing and/or repeated thawing and freezing which may lead to little loss of the RNA [134], despite other reports to the contrary, the RNA level remains stable during repeated thawing and freezing for about 8 cycles [135]. There is a possibility of a negative result by HCV-RNA testing during the convalescent period (the progression from the acute to the chronic phase) since the patient may lose HCV-RNA and remain negative in this period [130].

Superscript 9
The serological window phase, characterized by detectable HCV-RNA in the absence of HCV-Ab, has been estimated to be 60 days on average [145]. Some patients (50 - 70%) with acute infection who develop symptoms, will have detectable antibodies at that time, but 90% will have measurable antibodies after 3 months [118]. Thus, using a PCR assay, viremia can be detected within a few days of exposure to the virus and many weeks before elevation of transaminases and antibody titre [61-63].
The presence of HCV-RNA in the absence of HCV-Abs is strongly indicative of acute HCV infection, which will be confirmed by seroconversion (i.e., the appearance of HCV-Abs) a few days to weeks later [109].

Superscript 10
Acutely infected patients can also have both HCV-RNA and HCV-Abs at the time of diagnosis [109]. It is difficult, in this case, to distinguish acute hepatitis C from an acute exacerbation of chronic hepatitis C or an acute hepatitis of another cause in a patient with chronic hepatitis C [109].
The incubation period for newly acquired (acute) HCV infection ranges from two weeks to six months, with an average incubation period of 6 - 10 weeks [146-149]. The course of acute hepatitis C is variable, although elevations in serum ALT levels (often in a fluctuating pattern) are its most characteristic feature [137]. Normalization of ALT levels might occur and suggests full recovery, but this is frequently followed by ALT elevations that indicate progression to chronic disease [127, 151]. However, 50 - 70% of patients with acute infection who develop symptoms will have detectable antibodies at that time, but 90% will have measurable antibodies after 3 months [118].
Tests are not available to distinguish acute from chronic HCV infection [137]. However, the HCV-Ab IgG avidity index within the first 8 days following the onset of clinical symptoms may be useful in identifying actual acute HCV infection cases [36].
HCV-Ab IgM were reported in 50 - 93% of patients with acute hepatitis C and 50 - 70% of patients with chronic hepatitis C [151-153]. Therefore, HCV-Ab IgM cannot be used as a reliable marker of acute HCV infection [140]. However, the serial measurements of the HCV-Ab IgM titres based on at least three determinations from the fifth to the 15th day from the onset of the symptoms may identify patients with acute hepatitis C [153].
About 80% of acute HCV infections are asymptomatic [141] or have mild symptoms (fatigue, nausea, anorexia, vague abdominal discomfort, and vomiting) progressing to jaundice in about 25% of patients, less frequently than hepatitis B [140,141].
Most (70 - 90%) of infected people fail to clear the virus during the acute phase and become chronic carriers [127,133,134], that progress at variable rates (15 - 20%) to cirrhosis [137] and may (1 - 4%) develop to hepatocellular carcinoma [153]. About 5% of infected persons may die from the consequences of long term infection (liver cancer or cirrhosis) [128]. HCV-associated end-stage liver disease is the leading indication for liver transplantation in American adults [150,154].

Declaration of Interest:

Author declared no conflict of interests.

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