Hepatitis C virus

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THE MICROORGANISM AND ITS CLINICAL PRESENTATION

Hepatitis C virus (HCV) is an enveloped positive-sense RNA virus in the genus Hepacivirus. It belongs to the family of Flaviviridae. There is one single serotype but 8 genotypes differing more than 30% in nucleic acid sequence. Different genotypes cause the same disease, but differ in disease severity and in specific susceptibility to antiviral therapy. Some genotypes are ubiquitous (1a, 1b, 2b, 3a), while others are mainly restricted to specific areas and different methods of transmission (drugs, blood) [Gower 2014, Kuiken 2009, Borgia 2018].

The primary and most important feature of HCV infection is its clinical inapparentness: in most cases, symptoms of chronic liver infection only become apparent after decades [Strasak 2011]. 85-90% of acute HCV infections are asymptomatic [Irving 2008]. A minority develops non–specific flare-ups with abdominal pain, fatigue or flu-like symptoms. In less than 10%, signs of hepatitis such as transaminitis or icterus, lasting 3-12 weeks occur [Sagnelli 2014]. Without treatment, 10-40% clear the virus within 6-12 months [Micallef 2006, Scott 2007, Grebely 2014]. The majority of infected persons progress to chronic infection and will have detectable HCV-RNA and HCV-antibodies for years. Recent studies however, show that the prevalence of spontaneous clearance doubled over the past decade and reached 68.7% in 2017 [Seo 2020]. The immune response against HCV is not protective; both reinfections and superinfections occur frequently in high risk groups [LCI 2019]. Persons co-infected with HIV often have a mild infection but only 5-20% will clear the infection without treatment. The remaining 80-95% remain chronically infected getting early complications [LCI 2019]. In recent years, treatment of HCV with direct-acting antivirals (DAA) has become very effective; it has led to sustained virological response in more than 90% of treated persons [Feeney 2014, Pawlotsky 2014].

In chronic infections, extrahepatic symptoms are frequent and diverse; probably related to the persistent presence of HCV-RNA in blood. In 4066% of these chronic infections, the manifestations are related to immune- response (cryoglobulinic vasculitis in 60-90%of patients,

B-cell NHL, Arthralgia, Sicca syndrome) or autoantibodies (RF/ANF in 70% of patients) or inflammation (diabetes mellitus, glomerulonephritis in 10-20% of patients, or ischaemic heart and cerebrovascular disease) [Cacoub 2016, Younossi 2016].

COMPLICATIONS

- Fulminant infection does not occur!
- Up to 10-20% of chronically infected persons will develop liver cirrhosis after >20 years and 1-5% will develop hepatocellular carcinoma (HCC). Liver failure due to HCV infection is an important cause of liver transplantation worldwide. After transplantation 10-40% of patients (including patients with sustained viral response) will develop liver cirrhosis anew after 10-15 years due to HCV recurrence [Vinaixa 2013]. This may be less from the start of DAA treatments. In the Netherlands, annually 300-350 persons out of an average of 22,885 chronically infected individuals die from chronic HCV due to cirrhosis or HCC [Hofman 2016].

EPIDEMIOLOGY

HCV infections are ubiquitous; 3% of the world population is infected (=170 million). Humans are the only known reservoir for HCV. Worldwide, almost 400,000 persons die yearly from an HCV infection (112,500 in Europe). High prevalence regions (>3.5% of population) include Central Asia, SE Asia, N.Africa, low prevalence regions (<1.5%+) include Japan, N.America and S.America. Seroprevalence in Europe [ECDC 2018]: Germany 0.3-1%, France 0.8-0.9%, Italy 0.6-27.6% , Spain 0.4-1.5% and UK 0.4-1.2%. Seroprevalence in the Netherlands is 0.16%, 75% of infected persons are chronically infected [Koopsen 2018].
In the Netherlands, yearly 30-70 new HCV infections are diagnosed. 2/3 of the chronic infections are found in migrants coming from endemic regions [Urbanus 2011]. Acute infections occur mainly in MSM (men having sex with men) irrespective of their HIV satus. Transmission in IV drug users has been very low since "the syringe change" project [LCI 2019]. Dominant in Europe are HCV genotype 1 (61%) and genotype 3 (30%).

Hepatitis C is a blood-borne infection transmitted by blood-blood contact due to sharing of contaminated needles (in drug use, 60%), high-risk sexual contact (especially in MSM), mother-to-child transmission, contamination during circumcision/scarification/

tattooing with contaminated instruments, or by iatrogenic transmission of blood (products), transplantation of organs/tissue, and rarely through haemodialysis [LCI 2019, Ghany 2009]. In the Netherlands, the transmission risk by needle stick accidents is on average 1.9% [LCI 2019]. Globally 50.000 children [Yeung 2009] each year are born with HCV, infected during pregnancy or perinatally. The vertical transmission rate is approximately 5%. 1/3 of perinatally infected children clear the infection spontaneously, 2/3 need treatment. Success rates are variable depending of the genotype causing the infection (79% type 2-3, 18% type 1). Trials for children with new FDA approved DAA-based treatments are still ongoing [Indolfi 2019]. Maternally-derived antibodies are cleared only after 9-15 months. Therefore, babies born to mothers with an HCV infection should be tested for HCV-RNA in the 2th month of life, and an anti-HCV test at 12-15 months. If the anti-HCV test is positive after 12-15 months, it should be confirmed with HCV-RNA and ALT again.

DIAGNOSTIC TESTING

The clinical, viral and serological course are depicted in figure 1.

Techniques

- Anti HCV serology is highly automated and standardised using a multi-antigen format with immune dominant epitopes from HCV core antigen and non-structural proteins. Most currently used immuno-assays detect all immunoglobulins against HCV independent of isotype, also called total Ig or anti-HCV antibodies in general [Scheiblauer 2006, Alborino 2011, Colin 2001, Cadieux 2016, Villar 2015].
- 4th generation tests that combine the detection of anti-HCV antibodies with detection of hepatitis core antigen have an average time to positivity of 2-4 weeks in acute infection [Irshad 2013, Kamili 2012, Colin 2001, Cadieux 2016], but are not used in the Netherlands.
- RDT/POCT are available [Mane 2019, Tang 2017], but not used in the Netherlands. These are rapid tests that can be performed on saliva and on finger prick bloodsamples. Sensitivity and specificity of these
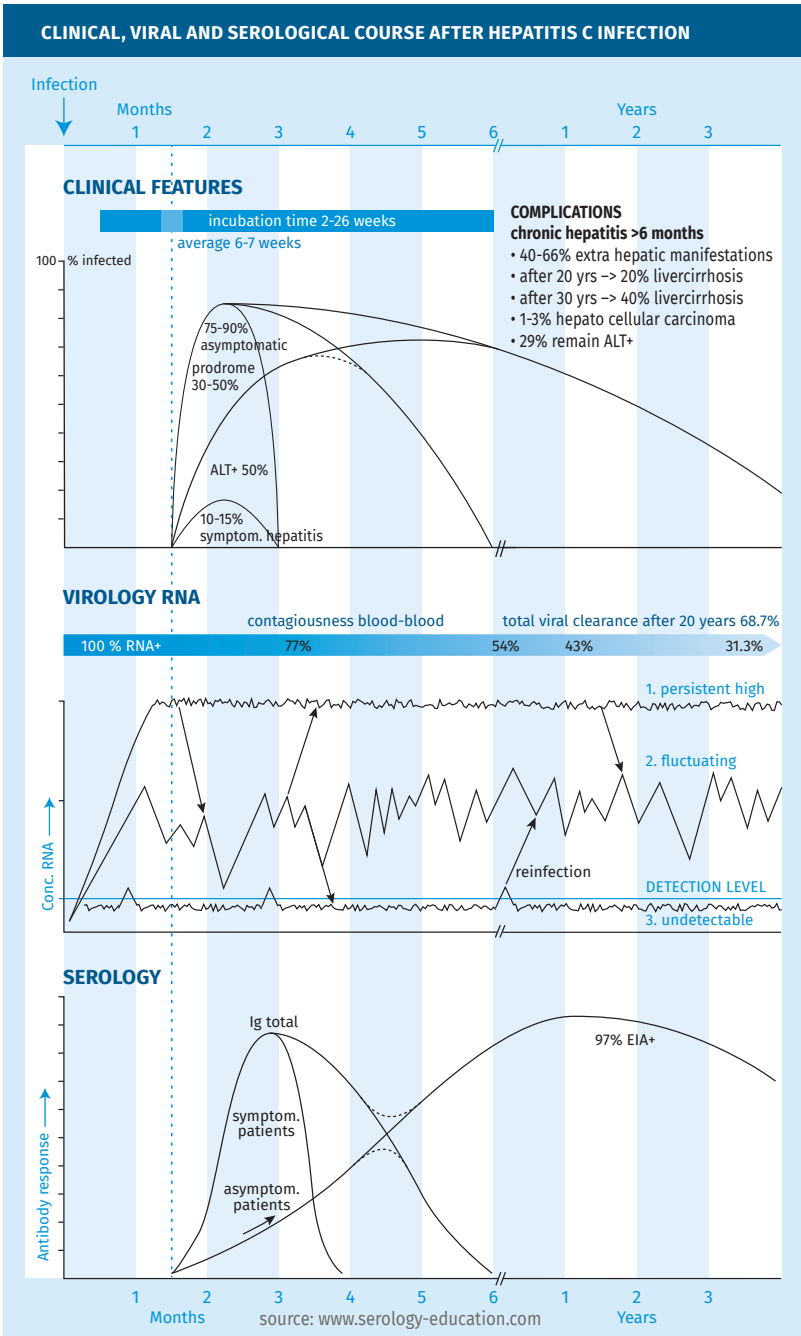
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* HCV core-antigen tests are easy, cheap and fast, and correlate well with HCV-RNA levels but have a lower sensitivity than PCR and therefore are used only for fast testing outside laboratories [Kuo 2012].

** Anti-HCV avidity tests [Sagnelli 2012, Coppola 2007, Villar 2015] distinguishing antibodies of low and high avidity to discriminate between recent (low) and chronic (high) infections are commercially available but are not used in routine diagnostics.

FIGURE 1.

CLINICAL, VIRAL AND SEROLOGICAL COURSE AFTER HEPATITIS C INFECTION

Infection

Months
1 2 3 4 5 6

Years
1 2 3

CLINICAL FEATURES

100 — % infected

incubation time 2-26 weeks
average 6-7 weeks

75-90% asymptomatic

prodrome 30-50%

ALT+ 50%

10-15% symptom. hepatitis

COMPLICATIONS
chronic hepatitis >6 months
• 40-66% extra hepatic manifestations
• after 20 yrs –> 20% livercirrhosis
• after 30 yrs –> 40% livercirrhosis
• 1-3% hepato cellular carcinoma
• 29% remain ALT+

VIROLOGY RNA

contagiousness blood-blood   total viral clearance after 20 years 68.7%

100 % RNA+   77%   54%   43%   31.3%

Conc. RNA

1. persistent high

2. fluctuating

reinfection

DETECTION LEVEL
3. undetectable

SEROLOGY

Antibody response

Ig total

97% EIA+

symptom. patients

asymptom. patients

1 2 3 4 5 6
Months

1 2 3
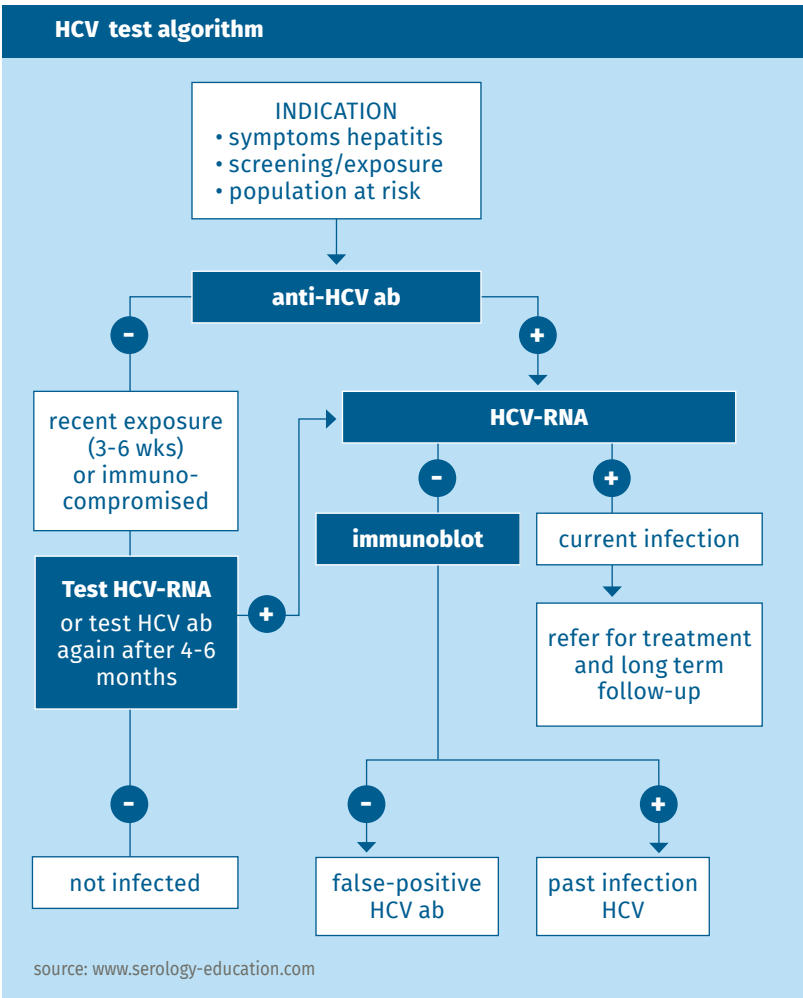Years

source: www.serology-education.com

The following studies were used to create the total figure: [Nikolaeva 2002, Kee 2012, Kondili 2002, Yuki 1995, Lau 1994, Mohd 2013, Stasak 2011, Villar 2015, Micallef 2006, LCI 2019, Aisyah 2018, Glynn 2001, Beld 1998, Scott 2007, Grebely 2014, Seo 2020]

rapid tests are lower than the automated EIA systems. A positive rapid test always needs confirmation with immunoassays or HCVPCR[Shivkumar 2012, Mane 2019]* **.

- The sole detection of IgM or IgA antibodies has no practical value [Gonzalez Quintela 2003].
- Immunoblot: Recombinant Immuno Blot Assay (RIBA) can be used as confirmation of low positive results or to confirm positive anti-HCV tests in low prevalence regions [Villar 2015].
- HCV-RNA /NAT to prove a current infection HCV-PCR test is obligatory.

FIGURE 2.

HCV test algorithm

INDICATION
- symptoms hepatitis
- screening/exposure
- population at risk

anti-HCV ab

-

recent exposure (3-6 wks) or immuno-compromised

+

HCV-RNA

-

immunoblot

+

current infection

Test HCV-RNA or test HCV ab again after 4-6 months

+

refer for treatment and long term follow-up

-

not infected

-

false-positive HCV ab

+

past infection HCV

source: www.serology-education.com

PRACTICAL USE OF SEROLOGY

Screening

Persons within risk-groups: anti-HCV antibody test. A positive test needs to be confirmed with HCV-RNA or, when HCV-RNA is negative, confirmation with immunoblot to rule out a non-specific reaction is necessary.

Screening in pregnancy and HIV/HBV positives should also include the test with HCV-RNA [Jhaveri 2018, LCI 2019].

Suspected infection in immunocompetent (chronic) persons

See table 1. Antibody levels decrease and are low in patients with chronic infection.

Suspected infection in immunocompromised persons

Immunocompromised persons have a delayed or even an absent anti-body response; therefore, an HCV-RNA test should be included to increase sensitivity.

INTERPRETATION OF SEROLOGY

The interpretation of anti-HCV results in an immunocompetent person is depicted in table 1.

TABLE 1.

anti-HCV	HCV-RNA	Confirmatory serology Immunoblot or other Immuno assay	Interpretation
Negative			Recent infection not excluded
Negative	Positive		Acute active infection (in immunno-compromised)
Positive or indeterminate	Positive		Present/active infect. (Recent, chronic or re-infection indistinguishable)
	Positive	Negative	Recent active infection
	Negative	Positive	Past resolved infection
	Negative	Negative	False positive screening assay

[Irving 2008, CDC-MMWR 2013, Boon 2020, Coppola 2007, Sagnelli 2012, Villar 2015, LCI 2019]

SENSITIVITY AND SPECIFICITY

There is a high agreement between commercially available immuno-assays and all have equivalent sensitivity and specificity of >99% being positive 4-6 weeks after infection.

Most infected individuals develop detectable antibodies within 3 months, but in immunocompromised persons, immune response can be delayed up to 12 months, or may even be absent. The sensitivity, though, declines rapidly in the first 6 months after infection.

Screening relies on antibody testing, but in persons who have cleared the infection antibodies persist low-level, therefore additional diagnostics, like HCV-RNA, are needed in order to confirm or rule out active disease. Testing in low prevalence regions like Western Europe using tests with 98% specificity, will yield more false-positive results than true-positive results [Cadieux 2016] (see table 2).

TABLE 2.

SENSITIVITY AND SPECIFICITY					
Reference with	**Sens %**	**Spec %**	**PPV**	**NPV**	**Prevalence**
Other antibody IA	70.4-99.5%	98.7-99.8%	63.3-98.6%	90.7-100%	2.3-38.8%
PCR vs antibody	61.0-81.8%	97.5-99.7%			

[Cadieux 2016]

• Immunoassays (IA) compared with other immunoassays and compared with reference to PCR do not measure technical specificity but solely activity of infection.

PITFALLS

• Patients with symptoms of hepatitis should always be checked for other causes of hepatotropic diseases, even when serology shows HCV antibodies, because chronic HCV infection most often progresses asymptomatic [Sagnelli 2014]!

• Positive predictive value in low prevalence populations is low: see general chapter on pitfalls [Ponde 2017, Schleiblauer 2006, Vo 2016].

• False-positive tests in low positive sera: see general chapter on pitfalls.

• Due to high occurrences of re- and super-infections of HCV, screening in patients with known previous infection should be performed with HCV-RNA.

• Seroconversion in HCV can be very late or absent especially in immunocompromised patients, follow up or HCV-RNA test will confirm results. Even in the follow-up results may remain negative!

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KEYWORDS

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