

not common, is found in the poly-transfused and intravenous drug users.

Therapeutic monitoring

The decrease in viral load under treatment is the direct prognosis of the long-term success of this treatment, at least for genotype 1.

A decrease in viral load exceeding 2 logs after 12 weeks of treatment, or even 4 weeks, points to a prolonged response. However, a reduction of less than 2 logs indicates a failure.

Genotypes 2 and 3, that are only treated for 24 weeks, are not concerned by this 12 week measure and not enough information is available for genotypes 4, 5 and 6 to be able to generalise these findings.

The therapeutic monitoring of treated hepatitis C is based, for all genotypes concerned, on the qualitative test for HCV RNA after 3 months, at the end of treatment and 6 months after the end of treatment to confirm the arrest of viral replication and the efficacy in terms of immediate response and prolonged response.

For patients infected with genotype 1, the monitoring also uses the measure of the HCV RNA viral load after 12 weeks of treatment for type 1 HCV, with the possibility of a possible suspension of treatment in case of no response after 12 weeks (lack of decrease in the viral load by at least 2 logs).

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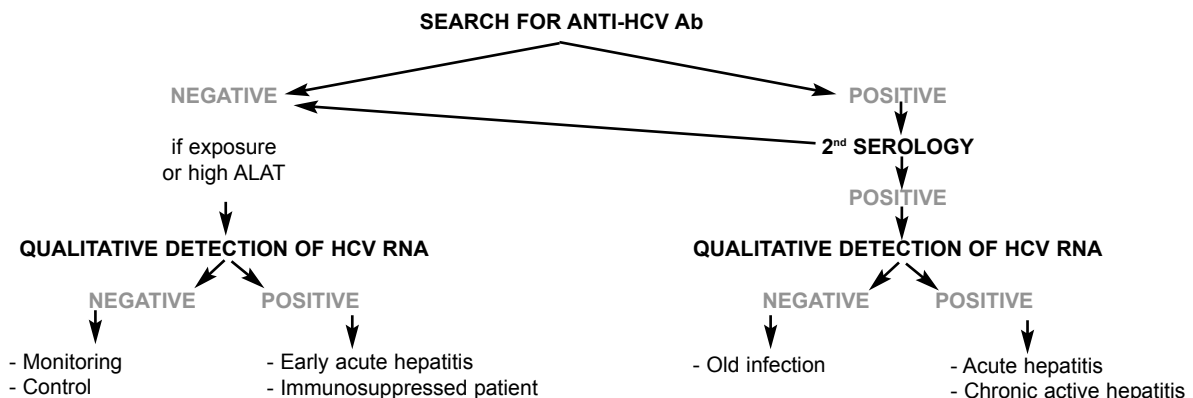
*Bibliography available upon request*

## Summary

### Biological Analyses and Indications

Indications	Analyses
Screening of anti-HCV Ab	Anti-HCV Ab by EIA
Confirmation of the positive reaction of anti-HCV Ab on a second sample	Control by EIA or Immuno-Blot
Initial assessment after discovery HCV positive	Qualitative detection of HCV RNA
Pre-therapeutic assessment	<ul style="list-style-type: none"> <li>- Hepatic tests : (ALAT, alkaline phosphatases, bilirubin, prothrombin level)</li> <li>- Hepatic fibrosis index (Fibrotest - Actitest)</li> <li>- Viral load</li> <li>- Genotyping</li> </ul>
Treatment monitoring	<ul style="list-style-type: none"> <li>- Viral load after 12 weeks</li> <li>- Qualitative detection of HCV RNA after 6 months, 12 months, 6 months after the end of treatment</li> </ul>
<ul style="list-style-type: none"> <li>- Length of treatment</li> <li>- Efficacy of treatment</li> </ul>	

### Decision tree





# HEPATITIS C

## Diagnosis and biological monitoring

### Editorial

*The hepatitis C virus (HCV) was described in 1989 as the main agent responsible for non-A and non-B hepatitis, after identification by molecular biology techniques (clonage and sequencing). Hepatitis C is a major public health problem in France, with about 400 to 500,000 carriers. It is one of the main causes of liver transplant in France and the United States. In France, one third of the mortality due to cirrhosis and primary liver cancer are hepatitis C related, representing about 4,000 deaths per year.*

*Biology reveals all of its importance in patient diagnosis and monitoring. For this reason, we have devoted the 58th issue of "La Lettre" to this topic.*

### The virus

The HCV is a virus with an envelope 55 to 65 nm in diameter. The envelope, bearing viral proteins E1 and E2 contains a capsid of icosahedral symmetry that protects a single stranded RNA with positive polarity of about 9.4 Kb. It belongs to the *Flaviviridae* family that includes the genus *Flavivirus*, with a great many arboviruses such as the Yellow Fever virus and the Dengue virus, and the genus *Pestivirus*, with viruses responsible for a great many animal diseases. HCV is classed in a third genus, that of *Hepacivirus*, of which it is the only representative.

HCV presents considerable genetic variability. This was used to define genotypes (80% homology) and

secondarily classify them in sub-types (90% homology). Six main genotypes have been described, numbered from 1 to 6, with a certain number of sub-types, identified by a lower case letter (1a, 1b, etc.).

### Epidemiology, Transmission

The transmission of HCV is parenteral in at least 60 to 70% of all cases. The two main modes of transmission are blood transfusion, before the systematic test of donors, and intravenous drug abuse, by the sharing of syringes or contaminated products. In France, the number of new cases is estimated at 4,000 to 5,000 cases per year, of which 80% are related to intravenous drug abuse.

The sexual transmission of HCV is possible although rare. Only 3 to 6% of the regular partners of HCV-infected subjects have infection markers.

Intra-family transmission seems possible considering the higher prevalence of anti-HCV Ab in family members of infected patients. The mechanism of this transmission is still not clear but may be related to a parenteral mode, by sharing objects potentially in contact with the blood, such as razor, toothbrush, scissors, etc.

Mother to child transmission has been demonstrated. It is common (20%) in case of HCV-HIV co-infection and rare (about 3%) in non HIV infected mothers. The higher the maternal circulating viral load in RNA HCV the greater the risk. The transmission seems to be perinatal.

Transmission by breast-feeding has not been demonstrated but can not be fully excluded.

A risk factor of infection has not been found in 30% or even more of the cases. However, most of these



patients seem to have been contaminated by parenteral route : transfusion or injection of blood products whose trace has been lost, nosocomial transmission (dental care, surgery, "aggressive" explorations), acupuncture, tattoo, etc. The populations with a high risk of infection are currently the polytransfused, especially before 1990, the transplanted, the haemophiliacs, the haemodialysed, the intravenous drug users and health care personnel.

## Clinics

After an incubation of 4 to 12 weeks, the HCV infection provokes often benign, most often asymptomatic, acute hepatitis (90% of the cases). There is a constant increase in transaminases, but the icteric forms are rare. There are no fulminant forms. Only 25 to 30% of acute hepatitis C patients recover spontaneously. This figure is higher in children. In about 70% of the cases, the acute hepatitis evolves towards chronicity, with a more or less rapid evolution towards fibrosis, associated with immune disorders : complex immune diseases (mixed cryoglobulinemia, membranoproliferative glomerulonephritis), presence of circulating autoantibodies, etc.

As opposed to other chronic viral infections, the severity of the infection is not related to the quantity of virus detected in the peripheral blood. The severity of the hepatic lesions is assessed by the histology of the hepatic needle-biopsy according to the Metavir classification, that associates a fibrosis score (F0 to F4) with a necrotico-inflammatory activity score (A0 to A3).

After 10 years of evolution, about 20% of the patients evolve towards cirrhosis. Contamination after the age of 40, excess alcohol and being a male are increased risk factors of evolution towards cirrhosis. About 3 to 5% of the patients suffering from HCV-related cirrhosis evolve towards hepatocellular carcinoma (HCC) each year. HCC does not seem to occur without preliminary cirrhosis.

Juvenile hepatitis C, of perinatal transmission, is relatively benign and may spontaneously heal in the first two years of life. The evolution towards cirrhosis is exceptional in the child.

## Diagnosis, Monitoring

### ■ Systematic screening for hepatitis C and confirmation

#### Test for Anti-HCV antibodies

Anti-HCV antibodies appear about 10 weeks after contamination, at the time of acute hepatitis or several weeks later.

The antibodies may diminish or even become non-detectable if there is a cure of the hepatitis C, following acute hepatitis (about 30% of the cases) or effective treatment.

This reduction seems to be a function of the length of

viral multiplication before its arrest and the individual immune response. Moreover, the antibodies may be absent with true chronic hepatitis C in patients producing few antibodies : haemodialysis, immunosuppressed patients. In this case, the only way to demonstrate the hepatitis C is to detect the virus.

The screening of anti-HCV antibodies necessarily requires the use of an ELISA type reagent.

A positive test should be confirmed with a second sample and a different reagent than the first one used. The purpose of this control is to eliminate possible, relatively frequent, false reactions obtained with all of the reagents commercialised. The control reagent can be another ELISA different from the first or an immuno-blot type test.

In general, strong initial positive reactions are almost always confirmed with a second test. A discrepancy between two ELISA (one limit or positive result and one negative result or two limit results) or between a positive ELISA and a negative or indeterminate immuno-blot should lead to the direct search for viral multiplication by qualitative PCR test in serum.

Hepatitis C screening is precisely described in the french ANAES recommendations of January 2001. It involves the following cases :

- subjects exposed to medical duties or behaviour involving a quantified and high risk of contamination (prevalence > 2%) ;
- subjects with an exposure factor involving a non quantified or low risk (prevalence < 2%).

### ■ Assessment of positive or indeterminate recently discovered HCV serology

#### Value of the Qualitative Detection of HCV RNA

The confirmation of an active HCV infection is based on the detection of HCV RNA in the serum using an amplification technique. The qualitative Amplicor HCV<sup>®</sup> test by Roche, with a sensitivity of 100 copies/ml or 50 IU/ml and TMA HCV<sup>®</sup> by Bayer, with a sensitivity of 50 copies/ml (25 IU/ml) are currently available. Other reagents are in the evaluation phase. These tests are carried out on serum, quickly separated and stored frozen (at -80°C for the long-term). The freezing of the serum has been shown to be able to wait for 72 hours at + 4 °C without provoking a quantitative alteration in the viral RNA although the initial separation should be as fast as possible to avoid a deterioration of this RNA.

The qualitative test for HCV RNA is in priority indicated in the initial assessment following the discovery of positive HCV serology, in the acute or chronic phase, to confirm or invalidate the viral replication, in parallel or successively to the confirmation of the initial positive serology reaction :

- the detection of RNA associated with a confirmed positive serology reaction attests replication of hepatitis C virus. The transaminases are most often high or subnormal but may also be strictly normal ;
- the detection of RNA associated with a negative or indeterminate serology is exceptional. It may correspond to acute hepatitis or chronic replicative hepatitis C within a context of immunosuppression. The transaminases are most often high or sub-normal ;

- the lack of RNA detection associated with an positive serology corresponds to past cured hepatitis C, without it being possible to date it. The transaminases are normal ;
- the lack of RNA detection associated with a positive serology almost always corresponds to a false positive screening reactivity, but may also be related to former cured hepatitis C where the antibodies are in the reduction phase. It is not possible to decide between these two situations.

The qualitative test for HCV RNA may also be indicated when acute or chronic hepatitis C is suspected without detectable antibodies.

*N.B.: quantification of circulating viral HCV RNA and genotyping are not recommended at the time of the initial assessment, outside of any therapeutic decision.*

### ■ **Treatment of hepatitis C : assessment and monitoring**

#### The means of treatment

According to the recommendations of the French consensus conference of February 2002, patients with a chronic HCV infection authenticated by the presence of viral RNA in the serum may benefit from a treatment.

The indications for treatment are based on histology of the liver, weighted by individual factors : change in the quality of life, co-morbidities and extra-hepatic manifestations. The motivation of the patient and those around him are also taken into account. These treatments involve adverse, sometimes disabling reactions.

A treatment associating IFN-PEG (Pegylated Interferon) and ribavirine is proposed in priority to :

- patients with moderate or severe chronic hepatitis (metavir F2 or F3) ;
- patients with cirrhosis (metavir F4) ;
- patients in relapse or not responding to monotherapy with Interferon ;
- patients transplanted for HCV-related cirrhosis or HCC.

These indications may be modulated according to the chronic consumption of alcohol, drug use, HCV-HIV co-infection, or the existence of psychiatric disorders.

- Bitherapy for 24 weeks is proposed for patients infected by a genotype 2 or 3.
- Bitherapy for 48 weeks is proposed for patients infected by a genotype 1, 4, 5 or 6.
- The treatment may be suspended after 12 weeks in case of a non-response for genotype 1.

The treatment of patients with minimum chronic hepatitis (metavir F0 or F1) or with normal transaminases is not recommended, except in case of extra-hepatic manifestations (vasculitis).

Acute hepatitis can benefit from an identical treatment with a high rate of success, without evolving towards chronicity.

#### Pre-therapeutic assessment

- **The assessment of hepatic fibrosis** is classically based on the hepatic needle-biopsy (HNP) and histology of the liver. There is no link between the quantity of circulating RNA found and the severity of the hepatic lesions noted by the histology of a HNP. Fibrosis and necrotico-inflammatory activity may also be assessed with indirect serum chemical markers or by calculation of the fibrosis or activity index. Among others, we can mention :

- the Fibrotest<sup>®</sup>, hepatic fibrosis index based on the serum concentrations in total bilirubin,  $\gamma$ -glutamyl-transpeptidases, haptoglobin, apolipoprotein A1 and  $\alpha$ -2-macroglobulin using validated techniques ;
- the Actitest<sup>®</sup>, an estimate of the necrotico-inflammatory action based on: the five previous parameters as well as ALAT transaminases.

50% of the hepatic biopsies could be avoided with the determination of these indexes, when well correlated with the histology.

- **The virology assessment** is based on the quantification of the circulating viral load in HCV RNA and on the determination of the HCV genotype.

#### 1) Quantification of the HCV RNA

It uses target amplification techniques (quantitative PCR Monitor HCV<sup>®</sup> by Roche) or signal amplification after hybridisation (branched DNA or bDNA, Versant HCV<sup>®</sup> by Bayer). The sensitivity thresholds of these two reagents are similar : 600 IU/ml for PCR and 615 IU/ml for branched DNA. The two techniques were standardised with respect to an international WHO standard and the results are provided in International Units per ml (IU/ml) and are directly comparable.

The quantification of the HCV RNA is indicated at the time of the pre-therapy virologic assessment, associated with the determination of the viral genotype. This measurement can be used to assess the risk of a good or poor response, and obtain a useful basic level for the monitoring of the treatment. A high viral load has been shown to be correlated with a higher risk of mother to child transmission. Moreover, a HCV RNA viral load exceeding 800,000 IU/ml is considered to be a factor of a poorer response and relapses are more common in the case of treatment of genotypes 1 in monotherapy with  $\alpha$ -Interferon.

#### 2) HCV serotyping - genotyping

**The serotyping test** determines the specificity of the circulating antibodies. This competition test for specific antigens is used to determine the type but not the sub-type. The sensitivity is only about 80%. This situation is common in the immunosuppressed or hemodialysed as well as in the immunocompetent. The main value lies in the determination of the viral type while the PCR is already negative at the beginning of treatment.

**The genotyping test** determines the viral type and sub-type. The determination of the sub-type is now only of epidemiological value. The genotyping techniques detect any co-infections by HCV of different types or sub-types. This situation, although