Serological and molecular biology screening techniques for HVC infection

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Abstract. This is a review of the main biological tests that can be requested by nephrologists to diagnose hepatitis C virus infection, together with a tentative specification of their indications and interpretation methods. This diagnosis is mainly based on serological tests (ELISA). The second and third generation tests contain, in addition to the NS4, proteins of the core and NS3 region. The latter two proteins provided great specificity and are antigenically well preserved in most virus types. They constitute the backbone of serological screening systems. In parallel with ELISA, analytical tests have been developed. In the form of immunoblots, they use proteins similar to those of ELISA and permit separate reading of antibody response to each protein. To achieve serological results, biologists must use at least two different techniques. Several strategies for using the various tests are thus available. However, serological diagnosis has its limitations: acute hepatitis most often precedes seroconversion; the results do not date the disease nor do they specify infectivity, evolution or recovery; seronegativity does not exclude a positive diagnosis with any certainty, especially in such populations as haemodialysis patients. To make up for the shortcomings of serological tests, direct viral detection can be achieved by genic amplification, most often by polymerase chain reaction in the best preserved part of the genome (5' non-coding end). PCR is currently becoming the real reference test for hepatitis C diagnosis. It is also possible to quantify viraemia and to determine the viral genotype. The relevancy of these two factors to therapy is controversial. Immunohistochemical study of liver biopsy specimens is still little used.

Introduction

The initial diagnostic methods specific for hepatitis C were developed 7 years ago. Significant progress has been made since in both the serology and the molecular biology of the virus. However, biological diagnosis of hepatitis C is still a difficult exercise: indications for the various tests are not easy to determine and test result interpretation is delicate. The introduction of a number of molecular biology tests whose epidemiological and/or clinical interest remains to be established has not simplified matters [1]. This is a review of the main biological tests available to nephrologists to prescribe, and we shall try and specify their indications and methods of interpretation.

Serological diagnosis

ELISA tests

There have been three generations of ELISA. The first generation tests contained only viral proteins from the NS4 region [2]. These tests produced false-positive results quite frequently, probably linked to the presence of yeast proteins (the substrate for recombinant protein synthesis). They also produced false negative results because they contained only NS4 of viral type 1a, a protein which differs significantly according to the HCV genotype [3,4]. Second generation tests contain, in addition to NS4 proteins, core and NS3 region proteins. These two additional proteins provide much greater specificity and are well preserved in most viral genotypes. They constitute the backbone of serological screening systems. Third generation tests contain one additional protein: NS5. This supplementation was decided after reports of seroconversion starting with NS5. This has not been confirmed and the addition of that protein is not decisively contributive. Some second generation tests perform just as effectively as those of the third generation.

Fourth generation tests are under development. They contain envelope protein epitopes. The first available results have not indicated any dramatic improvement of test sensitivity.

All these tests were developed from recombinant viral proteins produced in yeast or bacteria, or from synthetic peptides.

Detection of anti-core IgM is also possible [5]. Screening techniques of IgM directed against other viral specificities are not commercially available.

Immunoblots

In parallel with ELISA tests, analytical tests have been developed. In the form of immunoblots, they use
similar proteins as ELISA, and they permit separate reading of antibody response to each protein. These tests were initially proposed to make up for the poor specificity of early ELISA tests.

The following tests are available in France:

- Ortho: third generation ELISA, immunoblot (RIBA3)
- Abbott: third generation ELISA, 'confirmation test', anti-core IgM
- Murex: third generation ELISA, immunoblot
- Sanofi-Pasteur: second generation ELISA, immunoblot
- Innogenetics: immunoblot

**Legal obligations and strategies for using the tests**

To issue serological test results biologists in France are obliged by law to use at least two different techniques (except Blood Transfusion Centres). In practice, the most frequent testing strategies (protocols) are the following:

- one ELISA, then an analytical test if positive, or another ELISA if negative
- two ELISA and possibly an analytical test

The first strategy [7] may lead to false negative results (true positive not confirmed by immunoblot). The second one is more reliable and better adapted to large series, but it must be accompanied by a policy of 'non-confirmation' of strong positives by analytic tests to avoid excess cost.

We recommend systematic use of two ELISA for the first screening, to optimize sensitivity. Analytic tests should be reserved for sera producing weak positivity in at least one of the ELISA tests, or conflicting ELISA results. The second one is more reliable and better adapted to large series, but it must be accompanied by a policy of 'non-confirmation' of strong positives by analytic tests to avoid excess cost.

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**Limitations of serological diagnosis**

- Acute hepatitis most often precedes seroconversion
- Serological results (including IgM and analytical tests) do not date the affection, nor do they provide any accurate indication of infectiousness, evolutiveness and recovery
- Serological negativity does not rule out diagnosis with certainty [6], especially in such populations as haemodialysed patients [7]

**Molecular biology diagnosis**

**Detecting viraemia**

Direct viral diagnosis can be reached by gene amplification using one of the following techniques:

- reverse transcription followed by two PCR steps (primary and secondary steps): this is the standard technique [8], which is very sensitive but exposed to the risk of false positive results due to contamination
- reverse transcription followed by a single PCR step (Amplisor, Roche).
- amplification by NASBA system (Organon)
- gene amplification techniques use primers from the highly preserved region of the genome (5' non-coding end) [9-11]

'Ultra-sensitive' hybridization (bDNA Chiron), much less sensitive, can also be used.

PCR is becoming the reference test for hepatitis C diagnosis. Its price compares with that of an immunoblot. Great efforts have been made towards the standardization of the test. However, it is not included in the French nomenclature of medical biology. This test ideally should feature: (i) a system to prevent false positive results due to contamination; (ii) a screening system for enzyme inhibitors; and (iii) a system to assess the manipulation sensitivity, which is not included in the tests currently used.

It is usually accepted that any individual with positive serology and eligible for specific treatment should have a PCR. All haemodialysed patients with positive serology must also be tested in order to assess and control the risk of nosocomial transmission of the virus.

**Quantifying viraemia**

This can be done using various PCR techniques, the most simple and most used being that of 'fringe dilutions'. This rather is a semi-quantification.

The most accurate technique (unfortunately little sensitive) is molecular hybridization (bDNA).

The interest (disputed) of that titration (costly) would be the adjustment of interferon doses to the level of viraemia. This definitely is not a first intent test. It is reserved for very specific therapeutic protocols.

**Viral strain typing**

This can be achieved by characterizing the PCR products in various regions of the genome (Core, NS5, 5' NC) [12]. The non-coding 5' part, the target of screening PCR is often used for commodity. The diagnostic PCR products can be characterized by restriction or hybridization with specific probes (Innolipa test).

Typing can also be done by serotyping (in non-viraemic patients) (Murex test, currently being evaluated).

The main interest of the viral typing method is 'epidemiological', in the broader sense of the term. Knowing the genotype currently has no particular consequences on any given individual, even if certain types are known to be more pathogenic than others like 1b.
Immunohistochemistry

Immunohistochemical analysis of liver biopsy specimens with specific antibodies has been the subject of many interesting studies [13–15]. So far, no test has been marketed and no interpretation consensus has been found.

Conclusion

The basis of HCV screening remains serology, for reasons of cost and practicality. This screening has to be optimized by systematically using two different ELISA tests.

But serology can sometimes be wrong, and it is worth pondering the opportunity to systematically perform direct viral diagnosis by PCR in such populations as haemodialysis patients [16]. In their case, the risk of nosocomial transmission appears to justify that prescription entirely [17].

References