Clinician’s guide to diagnostic tests for hepatitis C virus infections

Following the discovery of hepatitis C virus (HCV), various diagnostic tests were introduced in rapid succession for use in blood banking and in the clinical arena. The plethora of tests and their interpretation have caused some confusion. This paper provides a brief review for clinicians concerning these tests and the pitfalls and limitations of their use. Three types of tests are discussed: second generation enzyme-linked immunoadsorbent assays (ELISAs), immunoblot procedures such as RIBA-II (Ortho) and MATRIX-HCV (Abbott Laboratories), and the polymerase chain reaction (PCR) for detection of viral genome.

Soon after the discovery of HCV, ELISA tests were developed to screen for antibody against recombinant peptides of the virus. The first generation tests were relatively insensitive due to inclusion of only one peptide coded by the nonstructural region of the virus. The second generation tests now in use include peptides derived from both the structural and nonstructural regions of the virus, and are 90% sensitive and over 99% specific in the detection of HCV infection (1,2). Within two to three weeks of onset of illness, 50% of patients develop antibody detectable by ELISA and more than 90% are positive by four to 11 weeks (3-5). In cases of acute hepatitis, the detection of anti-HCV by ELISA is highly predictive of HCV infection in the patient. However, in the screening situation, where prevalence of infection is low (as in blood donors), the positive predictive value of a reactive anti-HCV test is only, at most, 50%. Furthermore, the wide variability in the genomic constitution of HCV strains raises the possibility that presently available ELISA tests may not contain peptides carried by certain strains of virus, and therefore cannot detect them, ie, false negative tests (6). As knowledge of viral strains increases, even more sensitive tests will be developed. Finally, the problem of false positive ELISA results in patients receiving immunoglobulin therapy should no longer occur, because all plasma units from anti-HCV positive blood donors are now discarded.

Both Ortho Diagnostics Systems Inc and Abbott Laboratories have developed supplemental assays based on immunoblot technology for confirmation of the positive ELISA test. Recombinant peptides from both structural and nonstructural regions as well as control antigens are immobilized in discrete areas of a nitrocellulose membrane. The patient’s serum is put in contact with the nitrocellulose membrane and allowed to react with the immobilized peptides. Antibodies present in the sera which react are then detected by standard immunoenzymatic methods. A positive immunoblot test is highly predictive of viremia in the tested subject, documented either by PCR or by transmission of hepatitis C following transfusion of the subject’s blood (7-10). However, indeterminate results are problematic. Contrary to the situation with HIV serology, where indeterminate immunoblot results are nonsignificant in the overwhelming majority of cases, 20 to 25% of individuals with indeterminate results on RIBA-II are viremic (10,11). Therefore, indeterminate results for HCV infections must be interpreted cautiously and in the context of the patient’s clinical status and risk history.

The PCR tests for detection of HCV genome in sera of patients mostly utilize amplification of the 5’-noncoding region of the viral genome, which is highly conserved and permits detection of over 95% of all strains described to date (12,13). The advantages of PCR include detection of viremia directly and the ability to detect virus in liver biopsy samples. PCR can also be used to evaluate an infected patient’s response to interferon therapy and appears more sensitive than antibody tests for detection of HCV infection (1,13,14). This technique, however, is presently available only in research laboratories, is labour intensive, requires care to avoid cross-contamination leading to false positive results and is expensive. Eventually, this procedure is expected to be adapted for use in clinical laboratories, particularly for evaluation of individuals with indeterminate or negative
serological results and those undergoing interferon therapy. PCR will also be useful when antibody results are uninterpretable, such as in infants born to an infected mother where maternal antibody may persist for months, and in the immunocompromised host unable to raise any antibody response.

In summary, a number of tests are available for the exploration of HCV infection in patients. These tests are not perfect and require refinement. However, when used wisely, they are very helpful to the clinician. If interpreted inappropriately, they may create undue anxiety and result in investigations which are unnecessary and possibly deleterious to the patient.

REFERENCES

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