Chronic hepatitis C: new diagnostic tools and therapeutic strategies
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Chapter 12

GENERAL DISCUSSION

Based on the results of studies described in literature and our own results, guidelines for laboratory use and interpretation of currently applied HCV confirmatory assays are proposed. Furthermore, the implications for standard and future treatment are discussed.

I. STRATEGIES FOR CONFIRMATORY TESTING IN HEPATITIS C INFECTION

I.1. Detection of HCV in Serum

An important step in the diagnosis of HCV infection in asymptomatic individuals or patients with a reportedly positive anti-HCV serological test (ELISA), the presence of HCV antibodies should be confirmed by an HCV immunoblot assay. Using the results obtained with a third-generation recombinant immunoblot assay (RBIA-3), the following situations can be distinguished: a negative HCV-RIBA-3 result, indeterminate (HCV-RIBA-3 result called antigen reactive), and a positive HCV-RIBA-3 result (two or more antigens reactive). Not all antigens which are applied in RBIA-3 contribute equally to the reactivity of antibodies in HCV-RNA positive individuals. We found that the NS5 antigen contributed to a high rate of false positive reactions. Therefore, positive HCV-RIBA-3 reactivity consisting of reactivity of NS5 and one other antigen is tested separately from the other positive HCV-RIBA-3 reactivities in the flow diagram (Figure 1, paragraph 1.3).

To optimize the next generation of anti-HCV confirmation assays, one or two highly sensitive and specific HCV antigens should be added to the C22 and C53 antigens and more stringent interpretation criteria should be applied. This will lead to clearly distinguishable test results, i.e. true positive versus true negative. Additional HCV antigens which have been introduced up till now, i.e. E2 and NS5, do not improve sensitivity of anti-HCV detection nor sharpen the serological window phase in acute HCV infection.

I.2. Interpretation of HCV-PCR results

Detection of HCV-RNA has become an essential tool in the diagnosis of HCV infection and will soon become important in blood safety testing (chapter 2). Furthermore, determination of the HCV viral load with quantitative amplification techniques provides additional insight into viral kinetics during both the natural course of HCV infection and its treatment (chapter 6, 10). However, several problems must be considered when interpreting the results of qualitative and quantitative HCV-RNA tests.

Nucleic acid amplification methods are in principle highly sensitive and specific, but false positive and/or false negative test results can easily be produced. In recent quality control studies it was found that only 40-50% of laboratories reported reliable results with dna technique (chapter 4). In-house developed amplification methods, as well as commercially available amplification assays, produced false-positive and false-negative test results. False-positive results are usually due to contamination, while false-negative results are caused by suboptimal conditions with respect to sample transport and/or storage (chapter 7). With respect to amplification conditions, the choice of primers, false-negativity may also be caused by a low level of viremia (chapter 8). Most of these problems can be minimized by extensive standardization and validation of all test procedures (chapters 4, 5, 7).
antibodies and antibodies against shigella have been described (3, 52).

Two distinct cases of inhibitor development in relation to interferon therapy have been reported (5, 8). In one patient, who had mild haemophilia and had rarely been treated with factor VIII, interferon therapy had been discontinued for 2 months before inhibitors were detected. The other patient, who was still receiving interferon therapy when inhibitors were detected, did not have haemophilia but did have a multiple myeloma; his inhibitor had developed after autologous bone marrow transplantation. Factor VIII inhibitors may be associated with lymphadenopathy and this has led to concerns about concluding that interferon therapy caused the development of this inhibitor in this patient.

We show that the risk for development of inhibitors is not increased in patients receiving interferon compared with untreated patients. Inhibitor testing is usually done once or twice per year. The mean incidence of factor VIII inhibitors in our overall patient population is 29 per 1000 patient years (14). Most of these inhibitors are transient and produce no clinical complications; the cause of inhibitor development is usually unclear. We cannot exclude the possibility that the presence of inhibitors in our patients was caused by complications of interferon treatment. However, in our randomized, controlled trial, although the number of patients studied was limited, we found no evidence of enhanced inhibitor formation during interferon therapy.

REFERENCES

GENERAL DISCUSSION

Based on the outcome of studies described in literature and our own results, guidelines for strategically use and interpretation of currently applied HCV confirmatory assays are presented. Furthermore, the implications for standard and future treatment are discussed.

1. STRATEGIES FOR CONFIRMATORY TESTING IN HEPATITIS C INFECTION.

1.1. Interpretation of HCV-RIBA-3 results.
In samples from donors and patients with a repeatedly positive anti-HCV screening test (ELISA), the presence of HCV antibodies should be confirmed by an HCV immunoblot assay. Using the results obtained with the third-generation recombinant immunoblot assay (RIBA-3), the following situations can be distinguished: a negative HCV-RIBA-3 result, an indeterminate HCV-RIBA-3 result (one antigen reactive), and a positive HCV-RIBA-3 result (two or more antigens reactive). Not all antigens which are applied in RIBA-3 contribute equally to the detection of antibodies in HCV-RNA positive individuals. We found the NS5 antigen never and the C100 antigen only rarely essential for a positive anti-HCV result, whereas the C22 and C33 antigens were crucial for a positive result in most cases (chapter 3). Furthermore, the specificity of the third-generation antigens is not optimal (chapter 3)\(^1\). In particular the NS5 antigen contributes to a high rate of false positive results\(^2\)-\(^3\). Therefore, positive HCV-RIBA-3 reactivity consisting of reactivity of NS5 and one other antigen, is assessed separately from the other positive HCV-RIBA-3 reactivities in the flow diagram (figure 1; paragraph 1.3.).

To optimize the next generation of anti-HCV confirmation assays one or two highly sensitive and specific HCV antigens should be added to the C22 and C33 antigens and more stringent interpretation criteria should be applied. This will lead to clearly distinguishable test results, i.e. true positive versus true negative. Additional HCV antigens which have been introduced up till now, i.e. E2\(^4\)-\(^5\) and NS5, do neither improve sensitivity of anti-HCV detection nor shorten the serological window phase in acute HCV infection.

1.2. Interpretation of HCV-PCR results.
Detection of HCV-RNA has become an essential tool in the diagnosis of HCV infection and will soon become important in blood safety testing (chapter 2)\(^6\). Furthermore, determination of the HCV viral load with quantitative amplification techniques provides additional insight into viral kinetics during both the natural course of HCV infection and its treatment (chapters 6, 10). However, several problems must be considered when interpreting the results of qualitative and quantitative HCV-RNA tests.

Nucleic acid amplification methods are in principle highly sensitive and specific, but false positive and/or false negative test results can easily be produced. In recent quality control studies it was found that only 50-67% of laboratories reported reliable results with this technique (chapter 4)\(^7\). In-house developed amplification methods, as well as commercially available amplification assays, produced false-positive and false-negative test results. False-positive results are usually due to contamination, while false-negative results are caused by suboptimal conditions with respect to sample transport and/or storage (chapter 7), with respect to amplification conditions, or the choice of primers. False-negativity may also be caused by a low level of viremia (chapter 8). Most of these problems can be minimized by extensive standardization and validation of all test procedures (chapters 4, 5, 7).
One of the remaining problems in qualitative as well as quantitative HCV-RNA testing is the difference in amplification efficiency between the different HCV genotypes (chapters 5, 6). The choice of primers in most (commercial) HCV amplification assays is currently optimal for the majority of known HCV isolates. However, differences in the secondary structure of various HCV isolates may explain why differences in amplification efficiency occur (chapter 6). Optimization of the conditions for amplification in order to achieve a better denaturation of nucleic acid may improve and equalize amplification efficiency for the different HCV genotypes. These differences in amplification efficiency should also be taken into account when assessing results of clinical studies.

Figure 1:
Flow-diagram for confirmatory testing in hepatitis C infection.

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* in special situations (see §1.3)
** only including reactivity of NS5 + second antigen.
[2] HCV infection; this pattern is seldom seen, usually as C33 reactivity (chapter 3).
[4] HCV infection; this pattern has not been observed in our laboratory (chapter 3).
[7] Possible experienced, but resolved HCV infection. Assess risk factors for HCV infection before donor/patient counselling. At least two, yearly, follow-up visits are advised.
[9] No evidence for present or past HCV infection.
Another major problem in HCV-RNA testing is the difference in standardization between the various available assays: until recently, 100-fold apparent differences in HCV-RNA load could be observed when comparing the results of applying different quantitative assays to the same sample (chapter 4). Improvements have been made since the first WHO International Standard for HCV-RNA NAT assays was adopted.

1.3. HCV diagnostic flow diagram

Figure 1 shows a flow diagram which depicts the practical application of HCV (confirmation) testing.

Positive anti-HCV screening:
When, in an anti-HCV positive individual, the HCV-RIBA is negative (see fig 1; situation [1]), the anti-HCV screening result is most probably a false-reactive. When the HCV-RIBA is reactive and HCV viremia is detected using HCV-PCR (see fig 1; situation [2],[4],[6]) there is most probably an ongoing HCV infection (chapters 3, 8). It should be noted that HCV viremia is rarely detected in situation [2], i.e. indeterminate HCV-RIBA, or in situation [4], i.e. two-band reactivity of NS5 and one other antigen (chapter 3). Most of the subjects with pattern [2] or [4] are in the seroconversion phase of the infection or are immunocompromised (e.g. hemodialysis patients).

When HCV-RIBA is reactive, and HCV-RNA is negative, it is not possible to discriminate between false-positivity and established HCV infection (see fig 1; situation [3],[5],[7]). However, it has been shown that risk factors for HCV are significantly more often present in non-viremic blood donors with three- or four-band reactivity than in individuals with two-band reactivity (chapter 3). If antibodies against three or four antigens are present, an HCV infection is likely. As a consequence, when situation [3] or [5] is found in a patient or donor, and no risk factors for HCV infection are present, HCV immunoblot reactivity is likely due to false-reactivity. If risk factors are present, however, situation [3] and [5] are difficult to interpret.

In situation [7], the chances that the patient has experienced HCV infection are high, especially when risk factors are present. However, this pattern may represent a cleared infection. It has become apparent that in patients with a risk factor for HCV who have a positive HCV-RIBA without detectable HCV-RNA, i.e. situation [7], this pattern continues to be present in 90% of the cases, while in only 10% of the cases viremia is observed during a follow-up period of 2 years (chapter 8). Furthermore, sustained HCV-RNA negativity was found in 98% of the subjects who had been found to be HCV-RIBA positive and HCV-RNA negative at two consecutive yearly follow-up visits. In a recent 'look-back' study, blood products from viremic donors, but not from anti-HCV positive, HCV-RNA negative donors, caused HCV infection in recipients. Furthermore, in several studies HCV viremia, but not the presence of HCV antibodies, correlated with liver disease. Other studies have shown that liver disease improved histologically after sustained virological response in patients treated for chronic hepatitis C. Taken together, these findings suggest that an anti-HCV positive, HCV-RNA negative test result represents cleared HCV infection in the majority of the cases. Additional studies and longer follow-up of patients are necessary to confirm the validity of this interpretation.

Negative anti-HCV screening:
In donors or patients who are anti-HCV ELISA negative, usually no further HCV tests are performed. However, in special situations HCV viremia is present in seronegative individuals (see figure 1; situation [8]). This pattern can be observed during chronic HCV
infection in immunosuppressed patients (e.g. hemodialysis patients, HIV positive patients)\textsuperscript{10} and in acute HCV infection (e.g. serological window phase after HCV transmission). In these situations, HCV-RNA testing is obligatory to detect HCV infection and eventually start treatment.

Obviously, blood donated during the serological window phase is a significant risk for the safety of blood- and plasma products. In HCV infection, the serological window phase persists on average for about 82 days (54 to 192 days)\textsuperscript{17}, and is responsible for a risk of transmission of about 1 per 200,000 donations (1/530,000 to 1/97,000) (calculation based on results from voluntary blood donors from France during 1994-1996)\textsuperscript{18}. The window period in HCV infection can be ‘shortened’ to about 23 days when HCV-RNA detection methods are applied\textsuperscript{17}.

2. TREATMENT STRATEGIES IN HEPATITIS C INFECTION.

2.1 Standard HCV treatment algorithm.

Based on the results of several studies, a standard treatment algorithm has been proposed which, with some restrictions, can be used for routine out-patient care of HCV positive patients (chapters 9, 10). Using the IFN treatment schedule as depicted in figure 2, a sustained virological response rate of at least 40% can be achieved (chapter 10). The inclusion criteria for this treatment schedule are the same as those for clinical trials from which this standard treatment is derived: i.e. patients must be 18-70 year old with chronic hepatitis C (detectable HCV-antibodies and HCV-RNA > 6 months), elevated ALT levels, normal complete blood count, and without evidence of other hepatitis viruses, auto-immune hepatitis, decompensated cirrhosis, or other clinically significant systemic diseases. Follow-up procedures during and after treatment are also comparable to those described previously in the various relevant clinical trials (chapters 9, 10)\textsuperscript{19}.

Figure 2:
Flow-diagram for standard treatment of chronic hepatitis C.

\textsuperscript{174} This part of the algorithm can be performed as standard treatment in routine practice.
It is recommended that HCV positive patients, who do not comply with these criteria, are treated in specialized centres and/or in a trial setting, because it is not yet possible to define standard treatment for these patients. This also applies for those patients who do not, or only temporarily, respond to the standard treatment schedule as depicted in figure 2.

As the long-term prognosis of successfully treated HCV positive patients (sustained virological responders) is not yet known, these patients should be followed-up indefinitely.

2.2 New developments in HCV treatment.

A pressing problem for the successful treatment of HCV infection is the existence and/or development of therapy resistant viral strains. One of the possible solutions of this problem is the use of combinations of several antiviral drugs. At present, the most promising candidate treatment is Interferon-alpha (IFN) combined with Ribavirin. In particular, in patients with a high viral load this combination treatment has shown to reduce the relapse rate after cessation of treatment. It is expected that the combination therapy with IFN and Ribavirin will become the new standard therapy in the nearby future. A second candidate is to combine IFN and/or Ribavirin with Amantadine, an antiviral agent used for treatment of influenza A. Amantadine monotherapy for HCV infection has been associated with some positive results. Thirdly, antiviral agents especially designed to eradicate the hepatitis C virus (e.g. HCV protease inhibitors), which are currently under development, may be even more efficacious. The recently identified interaction between CD81 and HCV is probably also an important tool for future HCV treatment.

Another strategy to prevent the development of therapy resistant viral strains is to administer treatment more often and/or at a higher dose. In several studies, treatment with IFN at a dosage of 5 MU one to three times a day was efficacious, especially as induction treatment given for several weeks. Furthermore, in the near future it will be possible to detect and monitor quasispecies populations in plasma during treatment, and eventually to switch treatment strategies accordingly.

Viral as well as host factors should be considered in the treatment of HCV infection. A recent study of a large population of non-treated HCV positive patients showed that in patients infected with HCV below the age of 40, chronic liver inflammation progresses more slowly to fibrosis and cirrhosis than in patients infected over the age of 40. In patients infected with HCV below the age of 10 almost no progression occurs. Therefore, the age of onset of HCV infection may also influence the success of treatment and in some cases treatment may not be necessary.

In conclusion, optimal treatment of HCV infection will probably consist of combination therapy based on patient characteristics and virological data, and when these change, the treatment regime may have to be changed accordingly.

REFERENCES


