Chronic hepatitis C: new diagnostic tools and therapeutic strategies
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SUMMARY

In this thesis various aspects of diagnostic and therapeutic aspects of hepatitis C virus (HCV) are described. In the first part, a serological HCV confirmation assay is evaluated and results of the quality and standardization of currently used HCV amplification assays are presented. In the second part, the efficacy of an individualized, long-term antiviral treatment schedule is studied with the help of the special infection.

Chapter 2 is a review of literature on analyzable HCV-RNA amplification techniques, with the application for the diagnosis and research on hepatitis C infection. This chapter is based on the description methods in Chapter 1. In this part, the introduction to part I is mainly summarized (Chapter 2: Analyzable HCV amplification) of this thesis.

In Chapter 3, a third-generation enzyme-linked immunosorbent assay (ELISA) used from March 1993-May 1994 is compared with a second-generation RIBA (RIBA-2) used from March 1991-March 1993, using a validated HCV RNA PCR to establish plasma samples. In HCV RNA positive subjects, the sensitivity of RIBA-2 was significantly higher than that of RIBA-3, because of improved sensitivity of the antigens 253 and C100 and the number of indeterminate test results to RIBA-3 was significantly reduced. Increased nonspecific reactivity (isolated positivity to HCV RNA negative subjects) against the antigens 253 and C100 was observed in RIBA-3, but increased nonspecific reactivity against C22. The NS3 antigen was the least sensitive antigen of the RIBA-3 assay and produced a new group of nonspecific reactivities. Besides nonspecific isolated reactivity, nonspecific double-band reactivity was observed, especially in double-band reactivities other than C22 and C100. In the addendum to Chapter 3, four cases of RIBA-3 double-band positive/negative donors with a low probability of being infected with hepatitis C virus in the past, are presented.

In Chapter 4, an international quality control study for HCV RNA detection using the second EUROHEP HCV RNA reference panel is presented. Fifty-five percent of the 136 datasets contained false-positive or false-negative test results. Among laboratories with sufficient quality, up to 10,000-fold differences in sensitivity were observed. Furthermore, the detection efficiency for genotype 3 by commercial HCV RNA assays was found lower than when in-house-developed assays were used.

The results of the proficient laboratories participating in the second EUROHEP collaborative study for HCV RNA detection, were used to characterize the EUROHEP HCV-RNA genotype 1 and 3 plasma standards (Chapter 5). The 50% and 90% detection endpoints in the HCV genotype 1 dilution series were approximately 600 genome equivalents per ml (geq/ml) and 7750 geq/ml, respectively according to standards applied in the Chiron bDNA Quantiplex 1.0 assay. These bDNA values were close to those
SUMMARY

In this thesis various aspects of confirmational diagnosis of hepatitis C virus (HCV) and the treatment of chronic hepatitis C are described. In the first part, a serological HCV confirmation assay is evaluated, and results of the quality and standardization of currently used HCV amplification assays are presented. In the second part, the efficacy of an individualized, long-term interferon treatment schedule is studied with the help of the described diagnostic methods.

Chapter 1 (general introduction) summarizes the current knowledge on HCV structure and morphology, methods for detection of HCV infection with special emphasis on serological methods, and the natural history of HCV infection.

PART I

Chapter 2 is a review of literature of various available HCV-RNA amplification techniques, and their application for the diagnosis and research of hepatitis C infection. This chapter, together with the paragraph on serological methods in chapter 1, serves as an introduction to part I (confirmatory test methods in HCV infection) of this thesis.

In chapter 3 a third generation recombinant immunoblot assay (RIBA-3; used from March 1993-May 1994) is compared with a second generation RIBA (RIBA-2; used from March 1991-March 1993), using a validated HCV RNA PCR to establish plasma viremia. In HCV RNA positive subjects, the sensitivity of RIBA-3 was significantly higher than of RIBA-2, because of improved sensitivity of the antigens C33 and C100 and the number of indeterminate test results in RIBA-3 was significantly reduced. Reduced nonspecific reactivity (isolated reactivity in HCV RNA negative subjects) against the antigens C22 and C100 was observed in RIBA-3, but increased nonspecific reactivity against C33. The NS5 antigen was the least sensitive antigen of the RIBA-3 assay and produced a new group of nonspecific reactivities. Besides nonspecific isolated reactivity, nonspecific double-band reactivity was observed, especially in double-band reactivities other than C22 and C33. In the addendum to chapter 3 four cases of RIBA-3 double-band positive blood donors, with a low probability of being infected with hepatitis C virus in the past, are presented.

In chapter 4 an international quality control study for HCV RNA detection using the second EUROHEP HCV RNA reference panel is presented. Fifty-five percent of the 136 dataforms contained false-positive or false-negative test results. Among laboratories with sufficient quality, up to 10,000 fold differences in sensitivity were observed. Furthermore, the detection efficiency for genotype 3 by commercial HCV RNA assays was lower than when in-house developed assays were used.

The results of the proficient laboratories participating in the second EUROHEP collaborative study for HCV RNA detection, were used to characterize the EUROHEP HCV RNA genotype 1 and 3 plasma standards (chapter 5). The 50% and 90% detection endpoints in the HCV genotype 1 dilution series were approximately 600 genome equivalents per ml (geq/ml) and 7750 geq/ml respectively according to standards applied in the Chiron bDNA Quantiplex 1.0 assay. These bDNA values were close to those
calculated from statistical analysis of the limiting dilution PCR results. A 10-fold increase in sensitivity of the AMPLICOR HCV assay was observed when using a modified RNA extraction method. An international quality assessment program turned out to be a powerful instrument for characterization of viral nucleic acid plasma standards and validation of amplification assays.

In chapter 6 a quantitative nucleic acid sequence-based HCV RNA amplification assay (NASBA-QT) is evaluated and compared with the HCV bDNA assay (Chiron) and the HCV MONITOR assay (Roche). The quantitative detection limit was found to be $10^3$ copies per 100μl and the qualitative detection limit $10^{2.3}$ copies per 100μl. The HCV NASBA-QT assay was more than 10 times as sensitive as the bDNA assay and comparable in sensitivity with the HCV MONITOR assay. The quantitative results of the HCV NASBA-QT assay and the HCV bDNA assay were highly consistent.

In chapter 7 the influence of different anticoagulants and pre-amplification storage conditions on the stability of HCV RNA, as detected by the HCV NASBA-QT assay, is studied. It is demonstrated that clotted blood as well as EDTA or heparin anticoagulated blood can be used in this assay. After separation of plasma or serum, samples can be stored in lysis buffer for at least 15 months at -70°C, for at least 14 days at 4°C and for maximally 7 days at 30°C. Clotted whole blood can be stored at 4°C for 72 hours and EDTA-anticoagulated blood maximally for 48 hours; it is recommended to store whole blood samples at 4°C and to separate plasma or serum within 24 hours after collection.

In chapter 8 the prognosis of 48 hemophilia patients with an HCV-RIBA positive, HCV RNA negative test result, selected from an initial cohort of 363 hemophilia patients, is analyzed. It can be concluded that this test result in almost 90% of the cases reflects resolved HCV infection. It is advised to follow-up these patients yearly during 2 years. Thereafter, follow-up visits can be less frequent.

PART 2

Chapter 9 is a review of literature of hepatitis C treatment. This chapter serves as an introduction to part II (treatment in HCV infection) of this thesis.

In chapter 10 the efficacy of 6- and 36-month Interferon-alpha (IFN) treatment in patients with chronic HCV infection is described. The sustained virological response was 13% after 6 months (standard) and 40% (statistically significant higher) after 36 months (long-term) of treatment. Especially in patients with a virological breakthrough or relapse after standard treatment, long-term IFN retreatment was effective. In patients with a virological non-response during standard treatment, long-term prolonged treatment was not effective. Non-treated control patients showed a stable viral load during the observation period and no spontaneous clearance of HCV.

In chapter 11 the risk for development of antibodies to factor VIII (factor VIII inhibitors) in hemophilia patients with chronic hepatitis C is evaluated in conjunction with IFN therapy. Out of 21 patients receiving IFN therapy, two developed factor VIII antibodies, one also with clinical symptoms. Of the 14 nontreated control patients 3 developed
detectable factor VIII antibodies, without clinical symptoms. In the IFN group 3 patients were known with factor VIII antibodies compared to one in the control group. None of these four patients developed an increase of the anti-factor VIII titer or clinical symptoms. It can be concluded that in this patient group, long-term IFN treatment did not increase the risk of development of factor VIII inhibitors.

In chapter 12 (general discussion) the principal outcomes of the studies described in this thesis are discussed. Guidelines for use and interpretation of HCV assays and the implications for treatment are presented.