Quantitative Determination of Hepatitis C Core Antigen in Therapy Monitoring for Chronic Hepatitis C

Giovanna A. Moscato^a Gianluigi Giannelli^b Barbara Grandi^a Daniela Pieri^a
Ombretta Marsi^a Isabella Guarducci^a Irene Batini^a Emanuele Altomare^c
Salvatore Antonaci^b Alfonso Capria^d Giovanni Pellegrini^a Rodolfo Sacco^{c, d}

^aDepartment of Laboratory Medicine, Pisa University Cisanello Hospital, Pisa, ^bSection of Internal Medicine, Department of Internal Medicine, Immunology and Infectious Diseases, Bari University, Bari,

^cInstitute of Internal Medicine, Foggia University, Foggia, and ^dDepartment of Gastroenterology, Pisa University Cisanello Hospital, Pisa, Italy

Key Words

Chronic hepatitis C • Antiviral treatment • Therapy monitoring

Abstract

The correlation and kinetics of hepatitis C virus (HCV) RNA and HCV core antigen levels in chronic hepatitis C patients treated with pegylated interferon + ribavirin were evaluated in order to envision a combined use of the two assays in therapy monitoring. HCV core antigen levels by a chemiluminescent immunoassay (Abbott ARCHITECT) and HCV-RNA levels by branched DNA (bDNA) or real-time PCR have been evaluated on plasma specimens from 32 patients treated for chronic hepatitis C. An early virological response (undetectable levels of HCV-RNA 4 weeks after start of treatment) was found in 10/23 subjects (43.5%) followed up for 5 months or more. The response was linked to the HCV genotype (20% in genotype 1B vs. 61.5% in other genotypes; $p < 0.05$). HCV RNA and HCV antigen showed a good correlation ($r = 0.814$); HCV antigen was still detectable in 3 samples with undetectable (<615 IU/ml) RNA by bDNA, while no differences in clinical sensitivity were recorded in comparison with real-time

PCR. These findings suggest that HCV-RNA and HCV antigen may be used at different time points in order to tailor therapy monitoring to individual needs.

Copyright © 2010 S. Karger AG, Basel

Introduction

Hepatitis C virus (HCV) is a worldwide infection with a very high chronicization rate ($>70\%$), affecting almost 200 million people, and is still the leading cause of chronic liver disease in Italy [1]. Chronic hepatitis C may be treated by a combination of interferon and ribavirin. Monitoring of the virological response to treatment is usually performed by molecular assays that allow the quantitative determination of HCV-RNA in serum or plasma: a sustained response to therapy, indicated by a lack of detectable HCV-RNA for up to 6 months after the end of the treatment cycle, is achieved on average in 50–60% of patients, with marked differences linked to the HCV genotypes involved (i.e. $<40\%$ for genotypes 1 and 4, $>70\%$ for genotypes 2 and 3) [2]. The recommended timing for assessing the virological

response during therapy monitoring is at baseline, after 4 and 12 weeks, at the end of treatment (24 or 48 weeks according to the schedule) and every 3 months during the follow-up: additional controls at earlier time points (i.e. 1–2 days and/or 1–2 weeks after the start of treatment) have been suggested in order to achieve a higher predictive value for therapy outcomes [3–5]. However, since HCV-RNA testing is expensive and technically demanding, these alternatives for a closer follow-up are not adopted in practice. The recent availability of an immunoassay for the quantitative determination of HCV core antigen with a high sensitivity [6] has prompted us to carry on a preliminary evaluation of the performances of this new assay in comparison with two established methods for quantitation of HCV-RNA, in order to envision a potential combination of virological and serological testing in therapy monitoring.

Methods

Patients

We selected two groups of patients with chronic hepatitis C treated by a combination of pegylated interferon-2b plus ribavirin according to standardized protocols, that require higher doses and a longer period of administration (48 vs. 24 weeks) in patients infected by HCV genotype 1 or 4 and/or with baseline levels of HCV-RNA >200,000 IU/ml. All samples available from these patients were tested for HCV-RNA and alanine aminotransferase (upper normal limit 40 U/l) at the time of collection and then stored at –20° and tested in batch for HCV core antigen in May 2009.

Group A

Twenty-three patients admitted for chronic hepatitis C at the Department of Internal Medicine of University Hospital of Bari. These subjects were followed up for 5–13 months after the start of therapy (average 8 months). Testing time points were set at 1, 3, 6 and 12 months, HCV-RNA was determined by the branched DNA assay (Siemens Health Care) that has a lower limit of quantification at 615 IU/ml; the rapid virological response to antiviral treatment was established according to standard criteria [2]: (1) early virological response, if viremia became undetectable after 4 weeks of treatment; (2) complete response, if viremia was still detectable at 4 weeks but undetectable after 12 weeks; (3) partial response if viremia fell ≥ 2 logs at week 12, and (4) no response if viremia failed to decrease by at least 2 logs at week 12.

Group B

Nine patients admitted for chronic hepatitis C at the Department of Gastroenterology of the Cisanello Hospital in Pisa. These subjects are included in a controlled trial for the verification of very early virological response and prediction of outcomes by different treatment schedules: the time points for HCV-RNA testing are much closer than usual, being set at 1–2 days after the start of treatment, weekly for the first month and once a month after-

wards. HCV-RNA is being determined by a very sensitive real-time PCR assay (Roche TaqMan), with a lower limit of quantitation at 15 IU/ml of HCV-RNA.

Genotyping

HCV genotypes were determined by the INNO-LiPA commercial assay (Innogenetics, Ghent, Belgium).

Testing for HCV Core Antigen

We employed the recently developed Abbott ARCHITECT HCV antigen assay [6]: this is a fully automated, chemiluminescent microparticle immunoassay (CMIA) that incorporates an automated chemical pretreatment of the serum samples that allows the dissociation of antigen-antibody immune complexes and the lysis of viral particles, thus exposing the whole amount of HCV antigen present in the sample. This procedure allows the determination of HCV core antigen during all phases of HCV infection and not only during the early stages, when antibodies are not yet detectable [7]. The assay yields quantitative results, expressed either in femtomoles per liter or in picograms per milliliter: the limit of quantitation is 3 fmol/l (0.06 pg/ml) and the upper limit is 20,000 fmol/l, with the possibility of a 1:9 automated dilution that extends the assay linearity to 180,000 fmol/l.

Statistical Analysis

The coefficient of correlation between HCV-RNA and HCV antigen levels was carried out by the minimum-squares method (Spearman's *r* value).

Results

Group A

An early response to treatment was found in 10/23 subjects (43.5%); the response was linked to the HCV genotype (20% in genotype 1b vs. 61.5% in other genotypes; $p < 0.05$). The agreement between HCV-RNA and HCV antigen on the 105 samples available was 102/105, or 97.1%: three samples from three different patients were under the limit of detection by the bDNA and still positive for HCV antigen (0.06–0.11 pg/ml). As a consequence, in these 3 patients viremia became undetectable before the disappearance of HCV core antigen from serum (table 1).

Group B

The main goal in testing this group of subjects was to check the correspondence of HCV antigen with a highly sensitive assay for HCV-RNA: indeed, the qualitative correlation on the 61 specimens available was 100% (6 negatives and 55 positives by both assays). The clearance of HCV-RNA and HCV antigen was recorded at the same time (14 and 161 days after the start of therapy) in the 2 patients who have reached this outcome so far.

Fig. 1. Correlation between HCV-RNA (real-time PCR, Roche) and HCV antigen levels on 55 samples from nine patients with chronic hepatitis C treated by pegylated IFN and ribavirin.

Table 1. Summary characteristics of 23 patients followed up for 5–13 months during pharmacological treatment for chronic hepatitis C

Patient No.	ID	Geno-type	Virolog-ical response	Month of RNA negativization after the start of therapy	Month of HCVAg negativization after the start of therapy
1	17	1b	1	1	3
2	23	1b	1	1	1
3	7	1b	2	3	3
4	9	1b	2	2	2
5	21	1a	3	no	no
6	1	1b	3	6	6
7	6	1b	3	7	7
8	3	1b	4	no	no
9	10	1b	4	no	no
10	19	1b	4	no	no
11	11	2a/2c	1	1	1
12	22	2a/2c	1	1	1
13	4	2a/2c	1	1	1
14	16	2a/2c	2	3	3
15	20	2a/2c	2	2	2
16	5	2a/2c	2	2	2
17	8	3a	1	1	1
18	12	3a	1	1	3
19	14	3a	1	1	1
20	15	3a	1	1	2
21	18	3a	1	1	1
22	13	4	2	2	2
23	2	4c/4d	2	3	3

HCV-RNA has been determined by branched DNA. Virological response: 1 = early; 2 = complete; 3 = partial; 4 = none (see text for explanation).

Correlation between HCV-RNA and HCV Antigen

A quantitative result by both assays was obtained on 54 serum specimens assayed by real-time PCR, with viremia levels ranging from 131 to 6,720,000 IU/ml: in those samples the correlation between the two parameters was very high ($r = 0.799$; fig. 1). Nine samples (5 by real-time PCR and 4 by bDNA) showed HCV-RNA values between 100 and 1,000 IU/ml (mean: 616), and all were positive for HCV antigen with a mean value of 0.11 pg/ml: the limit of detection of the HCV antigen assays in respect to HCV RNA then appears lower than 500 IU/ml. Furthermore, the patterns of HCV-RNA and HCV antigen in patients who showed a response to treatment were very similar; an example (a patient with partial virological response) is shown in figure 2.

Discussion

The current serological diagnosis of HCV infection is based on the detection of anti-HCV antibodies and/or HCV RNA in serum or plasma, but positivity for anti-HCV does not allow a diagnosis of ongoing infection, as antibodies persist even for years after spontaneous or therapy-induced virus clearance. Conversely, in the early stages of infection, viremia precedes anti-HCV seroconversion by several weeks [7].

A number of studies have suggested that viral factors, such as viral genotypes, viral loads, amino acid sequence variations in the NS5A [8] and, as suggested very recently, in the core protein (aa #70/91) [9] are shown to be important for monitoring and predicting the outcome of the future therapy in chronic HCV infection. Assays for

Fig. 2. Profiles of HCV-RNA (log IU/ml) by RT-PCR and HCV antigen (log pg/ml) in a patient with chronic hepatitis C showing a partial virological response (decrease of 2.7 logs of HCV-RNA after 12 weeks of treatment). Numbers on the x-axis indicate the days after the start of treatment; the dotted line represents the cutoff of the HCV antigen assay (0.06 pg/ml).

HCV-RNA are now routinely used for the diagnosis of ongoing HCV infection as well as for therapy monitoring, whose outcomes are defined according to HCV-RNA results, since a 2-log reduction in the amount of HCV RNA or core antigen after the initial therapy is considered the minimum requirement for a virological response.

Assays for HCV core antigen have been proposed in the early '90s, mainly for detecting early infections [7]; a quantitative approach to HCV antigen determination has been proposed soon thereafter as an adjunct or even a replacement to HCV-RNA testing during the monitoring of chronic hepatitis C treatment [3, 4]. The main disadvantages of these earlier tests for HCV antigen were represented by the need of a manual pretreatment of the samples, to free the antigen from immune complexes and to release it from circulating virions, and the relative lack of sensitivity, even more evident as the new real-time PCR assays for HCV-RNA became available. Studies carried out with the earlier versions of HCV antigen assays have demonstrated that this antigen can be detected in the serum of most HCV-infected individuals concurrent with viremia and that the levels correlate quite well with HCV RNA levels [3, 4, 6].

Our preliminary results with the new, automated assay for HCV core antigen indicate that there is an excellent correlation between antigen and viremia and confirm the declared very good sensitivity of the serological method, that attains levels that are quite in accord with the experimental data from Schuettler et al. [10] who found a correspondence of 7,900 IU of RNA per pg of HCV core antigen in treated patients. In our opinion, this new method for the quantitative determination of HCV core antigen could become an alternative to HCV RNA for monitoring

antiviral therapy. More in detail, antigenemia could replace viremia in settings that still depend on less sensitive methods for HCV-RNA detection, such as the bDNA that was used in part of the patients we have described here. If a molecular assay for HCV-RNA with higher sensitivity, such as the current real-time PCR methods, is available, HCV antigen may represent a complementary method, especially whenever testing at more frequent time points is advisable in order to 'customize' antiviral treatment to the needs of the single patient [2, 4], or for alternative approaches with new drugs or different schedules, such as the recently proposed controlled therapy interruption [5].

The recent emphasis on the early kinetics of viremia and antigenemia as predictors of sustained response to treatment [4, 11, 12] may further enhance the usefulness of employing both a serological and a molecular approach for the evaluation of treated patients.

References

- 1 Sagnelli E, Stroffolini T, Mele A, Almasio P, Coppola N, Ferrigno L, Scolastico C, Onofrio M, Imperato M, Filippini P; Operative Units: The importance of HCV on the burden of chronic liver disease in Italy: a multicenter prevalence study of 9,997 cases. *J Med Virol* 2005;75:522-527.
- 2 Zeuzem S, Nelson DR, Marcellin P: Dynamic evolution of therapy for chronic hepatitis C: how will novel agents be incorporated into the standard of care? *Antiviral Ther* 2008;13: 747-760.
- 3 Takahashi M, Saito H, Higashimoto M, Atsukawa K, Ishii H: Benefit of hepatitis C virus core antigen assay in prediction of therapeutic response to interferon and ribavirin combination therapy. *J Clin Microbiol* 2005; 43:186-191.

- 4 Sasase N, Kim SR, KIM KI, Taniguchi M, Imoto S, Mita K, Hotta H, Shouji I, El-Shamy A, Norifumi Kawada N, Kudo M, Hayashi Y: Usefulness of a new immunoradiometric assay of HCV core antigen to predict virological response during PEG-IFN/RBV combination therapy for chronic hepatitis with high viral load of serum HCV RNA genotype 1b. *Intervirology* 2008;51(suppl 1):70–75.
- 5 Lake-Bakaar G: The effect of controlled therapy interruption in chronic HCV infection: enhanced host immune response? A hypothesis. *J Clin Virol* 2009;44:149–151.
- 6 Morota K, Fujinami R, Kinukawa H, Machida T, Ohno K, Saegusa H, Takeda K: A new sensitive and automated chemiluminescent microparticle immunoassay for quantitative determination of hepatitis C virus core antigen. *J Virol Methods* 2009;157:8–14.
- 7 Icardi G, Ansaldi F, Bruzzone BM, Durando P, Lee S, De Luigi C, Crovari P: Novel approach to reduce the hepatitis C virus (HCV) window period: clinical evaluation of a new enzyme-linked immunosorbent assay for HCV core antigen. *J Clin Microbiol* 2001;39:3110–3114.
- 8 El-Shamy A, Nagano-Fujii M, Sasase N, Imoto S, Kim SR, Hotta H: Sequence variation in hepatitis C virus nonstructural protein 5A predicts clinical outcome of pegylated interferon/ribavirin combination therapy. *Hepatology* 2008;48:38–47.
- 9 Akuta N, Suzuki F, Hirakawa M, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Kumada H: Amino acid substitutions in the hepatitis C virus core region of genotype 1b affect very early viral dynamics during treatment with telaprevir, peginterferon, and ribavirin. *J Med Virol* 2010;82:575–582.
- 10 Schuettler CG, Thomas C, Discher T, Friese G, Lohmeyer J, Schuster R, Schaefer S, Gerlich WH: Variable ratio of hepatitis C virus RNA to viral core antigen in patient sera. *J Clin Microbiol* 2005;42:1977–1981.
- 11 Fujino T, Nakamuta N, Aoyagi Y, Fukuizumi K, Takemoto R, Yoshimoto T, Miyahara T, Harada N, Sakai H, Nakashima M, Enjoji M: Early decline of the HCV core antigen can predict SVR in patients with HCV treated by pegylated interferon plus ribavirin combination therapy. *J Dig Dis* 2009;10:21–25.
- 12 Durante-Mangoni E, Zampino R, Portella G, Adinolfi RE, Utili R, Ruggiero G: Correlates and prognostic value of the first-phase hepatitis C virus RNA kinetics during treatment. *Clin Infect Dis* 2009;49:498–506.