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FULL PAPER

Hepatitis C Virus Core Antigen (HCVAg): an affordable assay to monitor the efficacy of treatment in DAAs era

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SUMMARY

Hepatitis C virus (HCV) Core Antigen (HCVAg) and HCV-RNA were tested in 962 plasma/serum samples from 180 patients during Direct Antiviral Agents (DAAs) treatment and at follow-up. One hundred and eighty individuals were included: 71% carried advanced fibrosis and 43% were treatment-experienced. A Sustained Virological Response (SVR) was achieved in 166/180 (92%) individuals: 96/102 (94.1%) naïve and 70/78 (89.7%) treatment-experienced (p=0.20). The baseline median levels of HCV-RNA and HCVAg were not significantly different between individuals achieving SVR (5.92 x 10^5 IU/mL, IQR 5.4-6.4, and 3,417 fmol/L, 2,900-3,795) and those without SVR (6.06 x 10^5 IU/mL, 5.63-6.57, and 3,391 fmol/L, 2,828-4,077).
The HCV-RNA vs. HCVAg assays results showed a fair correlation with an overall moderate qualitative agreement (kappa=0.52). Among treatment-failed individuals, at failure 100% of the assays results were positive for both techniques, with HCV-RNA median value 3.09 x 10^5 IU/mL (2.10-29.09) and HCVAg median value 1570.28 fmol/L (360.15-9317.67). Undetectable HCV-RNA at EOT showed sensitivity 54%, specificity 100%, negative predictive value (NPV) 93% and positive predictive value (PPV) 100%. Undetectable HCVAg at EOT showed sensitivity 74%, specificity 100%, NPV 97% and PPV 100%.
The operative and economic advantages of the HCVAg support the alternative use of HCVAg to monitor DAAs treatment outcome.

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INTRODUCTION

Hepatitis C virus (HCV) infection is one of the leading causes of chronic liver disease worldwide. The global prevalence of people living with active HCV infection is estimated to be about 1% (95% uncertainty interval 0.8-1.1), corresponding to 71.1 million (62·5-79·4) viremic infections (The Polaris Observatory HCV Collaborators, 2017). The long-term natural history of chronic HCV infection is highly variable but is associated with substantial morbidity and mortality, especially due to liver-related complications, including cirrhosis, liver failure and hepatocellular carcinoma (HCC), and contributes to the development of several extrahepatic manifestations (EASL, 2020). However, chronic HCV infection can remain silent for several years in a substantial proportion of individuals (WHO, 2018).
Since the 1990s, the goal of antiviral therapy has been to prevent these complications by achieving HCV eradication, verified by undetectable HCV-RNA 12 or 24 weeks after treatment, defined as sustained virologic response (SVR) (EASL, 2020; Vermehren

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J, 2018). An SVR corresponds to an eradication of the HCV infection, with a very low chance of viral relapse.

In the last few years, the availability of different Direct Antiviral Agents (DAAs) with very high efficacy in HCV eradication has improved the clinical prognosis of chronically infected individuals (Vermehren J., 2018). Globally, an SVR by DAAs is achieved in more than 95% of HCV-infected individuals after relatively short DAAs treatments (8-24 weeks) (EASL, 2020; Vermehren J., 2018).

The screening of HCV infection is based on the use of serologic assays detecting specific anti-HCV antibodies; virological diagnosis confirmation by HCV-RNA detection is required (EASL, 2020; WHO, 2018). Monitoring of DAAs efficacy is based on detection of HCV-RNA levels in serum or plasma during treatment, without stopping rules, and, particularly, during follow-up to define SVR.

HCV core antigen (HCVAg) is a viral protein released into the plasma during HCV assembly. Despite a lower level of analytical sensitivity, measurements of HCVAg levels in serum or plasma are recommended as an indirect active marker of viral replication, alternative to HCV-RNA, to improve affordability to diagnose viremic infection and to assess HCV treatment response (EASL, 2020; WHO, 2018). An assay with a limit of detection of 3000 IU/mL or lower would be acceptable and would identify 95% of those with viremic infection, based on available data (WHO 2018). Recently and in different settings, HCVAg has demonstrated good sensitivity and specificity to detect active HCV infection, especially when the viral load exceeds 3000 IU/mL, and to monitor efficacy of DAAs treatment (Aghemo A., 2016; Loggi E., 2017; Chevaliez S., 2018; Freiman J.M., 2019).

In DAAs era, early diagnosis, wide access to treatment and prompt detection of rare therapeutic failures are priorities in both high-income and low-income countries, and therapy monitoring should be adapted to guarantee an affordable economic and organizational model.

The aim of the study was to compare the accuracy of HCV-Ag to HCV-RNA during interferon-free DAAs treatments and for detection of therapeutic failure, in different real-life settings in Italy.

MATERIALS AND METODS

Population

This retrospective study was conducted at the Infectious Diseases Unit of Azienda Ospedaliero-Universitaria Senese (Siena, Italy) and at ITEC Outpatient Clinics of Azienda Ospedaliero-Universitaria di Bologna (Bologna, Italy). The 2 different clinical centers contributed an almost equal number of individuals (89 from Siena and 91 from Bologna) treated with different DAAs interferon-free regimens from June 2013 to July 2017. Partial results from some individuals were included in a previously monocenter published research (91 of 96) (Loggi E., 2017). As opposed to the previous report, for this multicenter study we selected a doubled population, including only DAAs interferon-free regimens. In order to better explore the possible role of HCVAg in real-life settings, HCVAg results were compared with 2 different RT-PCR-based methods, according to routine tests performed in 2 different clinical centers.

Briefly, the inclusion criteria were: adult age (≥18 years), chronic HCV infection confirmed by serum HCV-RNA using an RT-PCR-based method and fulfilling the eligibility criteria for treatment with only interferon-free DAAs regimens according to the recommendation of the Italian Medicines Agency (Agenzia Italiana del Farmaco-AIFA) at that time.

The population included:

1. individuals with advanced liver disease (METAVIR stage F3/4) or clinical cirrhosis;
2. individuals with HCV recurrence after liver transplantation;
3. individuals with extrahepatic manifestations, such as cryoglobulinemia and its correlated complications, regardless of the stage of hepatic fibrosis.

The treating physicians prescribed DAAs, treatment duration and use of weight-based ribavirin (RBV), according to current guidelines and individuals' clinical condition. The study was approved by the Ethics Committee of each clinical center and written informed consent was obtained from all individuals before participation. The study was performed in accordance with the ethical guidelines of the Declaration of Helsinki (7th revision) and with the International Conference on Harmonization Good Clinical Practice guidelines.

HCV testing

HCVAg and HCV-RNA were tested in plasma or serum samples of individuals at baseline, during direct-antivirals agents (DAAs) treatment at week (W) 2 and/or 4, at the end of treatment (EOT) and after 12 weeks and/or 24 W of follow up. The HCV-RNA levels were measured with the COBAS AmpliPrep/COBAS TaqMan HCV Quantitative Test, version 2.0 (Roche; lower limit of quantitation, LLQ =15 IU/mL) or with Abbott Molecular RealTime HCV (LLQ=12 IU/mL), depending on the system in use at each Laboratory. HCV-RNA was considered positive if the level was equal to or higher than LLQ (≥15 IU/mL or ≥12 IU/mL, respectively), whereas detectable but not measurable HCV-RNA levels were reported as <15 IU/mL or <12 IU/mL and scored as a gray zone. Undetectable HCV-RNA results were considered negative. The HCV genotype and subtype were determined using the Bigdye terminator v3.1 cycle sequencing kit AB or by Siemens VERSANT HCV Genotype INNO_

LiPA 2.0. A frozen aliquot from each sample was retrospectively assayed for HCVAg by the ARCHITECT automated immunoassay (Abbott Diagnostics, Wiesbaden Germany), with a limit of detection of 3 fmol/L. For HCV-Ag non-reactive results were defined by levels below 3.0 fmol/L, "gray zone" by levels between 3.0 and 9.9 fmol/L, and positive results by levels equal to or above 10 fmol/L with a dynamic range of the test from 3 to ±20,000 fmol/L and an extension to 180,000 fmol/L by automated dilution.
The SVR was defined as the undetectability of HCV-RNA at 12 and or 24 weeks after completing treatment (SVR 12/24). Post-treatment relapse (R) was defined as confirmed detectable HCV-RNA above LLQ during follow-up in individuals with undetectable HCV-RNA at the end of treatment (EOT). Viral breakthrough was defined as a ≥1 log10 IU/ml increase from the nadir of HCV-RNA or when HCV-RNA was above LLQ after becoming undetectable during the treatment.

Statistical analysis

Descriptive statistics [number, proportion, median, interquartile range (IQR)] were used to describe the baseline characteristics of patients. Categorical variables were compared between groups using the Chi-square test or Fisher's exact test, as appropriate. Continuous variables were compared using the Mann-Whitney U test. To assess the correlation between HCVAg and HCV-RNA, non-parametric Spearman correlation and kappa statistic were performed. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of HCVAg were assessed using HCV-RNA as the gold standard. Only p values of <0.05 were deemed significant. All analyses were performed using SPSS for Windows (Statistical Package for the Social Sciences, version 21.0, Armonk, New York, NY, USA) and GraphPad Prism, version 5.

RESULTS

Study population

A total of 1,002 samples from 180 individuals treated with different interferon-free DAAs regimens were analyzed. Detailed demographic and individuals' clinical characteristics are reported in *Table 1*. An advanced fibrosis (F3-F4) prevailed in about 71%. In 32 individuals the data on fibrosis stage were missing because they were treated after liver transplantation or for extrahepatic HCV-related manifestations.
HCV genotype 1, either 1a, 1b or undetermined, was prevalent, being identified in 123/180 (68.3%) individuals, while the prevalence of genotypes 2, 3 and 4 was 8.3%, 15.6% and 7.8%, respectively. The DAAs regimens, reflecting drug availability during the study conduction, were distributed as follows: 119 (66%) individuals started a 12-week treatment schedule, of which 76 naïve and 43 treatment-experienced individuals, while 61/180 (44%) individuals underwent a 24-week treatment schedule, of which 26 naïve and 35 treatment-experienced individuals. The shorter schedule was then administered to 74.5% of naïve individuals compared to 55.1% of individuals who failed previous IFN-based treatments (p<0.05). According to current recommendations, we confirmed that 172/180 (96%) individuals received an optimal duration therapy while 10/180 (5%) prematurely discontinued the treatment.

Table 1 - *Baseline demographic and clinical characteristics of patients (n=180).*

Age, median (IQR)	59 (51-69)
Male gender, n (%)	108 (60)
Treatment experienced, n (%)	78 (43)
HCV-RNA IU/ml, median (IQR)	5.97 x10^5 (5.42-6.40)
HCVAg fmol/L, median (IQR)	3,397 (2,903-3,809)
HCV genotype, n (%)	
1a	37 (20.6)
1b	85 (47.2)
1 undetermined	1 (0.5)
2a/c	15 (8.3)
3a	28 (15.6)
4a/c/d	14 (7.8)
HIV coinfected	20 (11)
HBV coinfected	0
Liver fibrosis stage, n (%)	
F0-F2	19 (11.5)
F3	29 (16.1)
F4	100 (55.5)
Unknown	14 (16.9)
DAAs treatment regimen, n (%)	
SOF + SIM ± RBV	44 (24.5)
SOF/LDP ± RBV	42 (23.3)
OBV/PTV/r ± DSV ± RBV	36 (20.0)
SOF + RBV	36 (20.0)
SOF + DCV	20 (11.1)
GRZ/EBV	2 (1.1)

Ag, antigen; DCV, daclatasvir; DSV, dasabuvir; EBV, elbasvir; GRZ, grazoprevir; HCV, hepatitis C virus; LDP, ledipasvir; OBV, ombitasvir; PTV, paritaprevir; r, ritonavir; RBV, ribavirine; SIM, simeprevir; SOF, sofosbuvir.

Sustained virological response

A sustained virological response was achieved in 166/180 (92%) individuals (intention to treat analysis): 96/102 (94.1%) naïve and 70/78 (89.7%) treatment-experienced individuals (p=0.20). Relapses were diagnosed in 14 individuals after 4/12 weeks from treatment completion. According to genotype (G), SVR was achieved in 33/37 (89.2%) G1a carriers, 80/85 (94.1%) in G1b, 12/15 (80%) in G2, 26/28 (92.9%) in G3 and in all G1 undetermined and G4 carriers.
The lower SVR rate observed for G2 carrier individuals could be related to suboptimal first-generation DAAs regimens, SOF + RBV in 13 out of 15.

According to the DAAs regimen, SVR rate was 100% in Ombitasvir (OBV)/Paritaprevir (PTV)/Ritonavir (r) ± Dasabuvir (DSV) ± RBV and Grazoprevir GRZ)/Elbasvir (EBV), 97.6% in Sofosbuvir (SOF)/Ledipasvir (LDP) ± RBV, 95% in SOF + Daclatasvir (DCV) ± RBV, 88.6% in SOF + Simeprevir (SIM) ± RBV, and 80.6% in SOF±RBV. Among individuals who completed DAAs regimens as prescribed, 160/172 (93%) achieved SVR vs 6/8 (75%) among those who prematurely stopped the treatment (p=0.06).

HCV-RNA and HCVAg levels

All individuals were positive for HCV-RNA and HCVAg at the beginning of treatment. The baseline median levels of HCV-RNA and HCVAg were not significantly different between individuals achieving SVR (5.92 x 10^5 IU/mL, IQR 5.4-6.4, and 3,417 fmol/L, IQR 2,900-3,795) and those without SVR (6.06 x 10^5 IU/mL, IQR 5.63-6.57, and 3,391 fmol/L, IQR 2,828-4,077).

A direct comparison of HCV-RNA vs HCVAg was performed on 962 samples. The results of the two assays showed a fair correlation at baseline and during monitoring (Spearman; r=0.80 and 0.75, respectively) with an overall moderate qualitative agreement (kappa= 0.52) (*Figures 1-2*); all 63 samples positive for HCV-RNA and negative for HCVAg had <1,000 IU/mL. Among treatment failed individuals, 100% of the assays results were positive for HCV-RNA and reactive or in gray-zone (2 cases) for HCVAg 12 weeks after completing treatment, with HCV-RNA median value 3.09 x 10^5 IU/mL (IQR 2.10-29.09) and HCVAg median value 1570.28 fmol/L (IQR 360.15-9317.67). Among the 166 individuals achieving SVR, only 2 individuals showed HCVAg value in grey zone (3.22 and 6.81 fmol/L) at EOT, while all were negative for HCV-RNA. The negativity rates for HCV-RNA in individuals achieving SVR vs individuals without SVR were 42% and 21% at W4, 93% and 100% at the EOT, respectively (*Figure 3*).

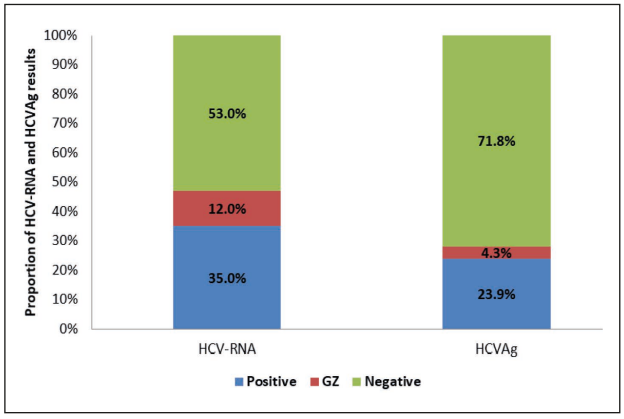


Figure 1 - *Qualitative comparison between HCV-RNA and HCV-Ag.*

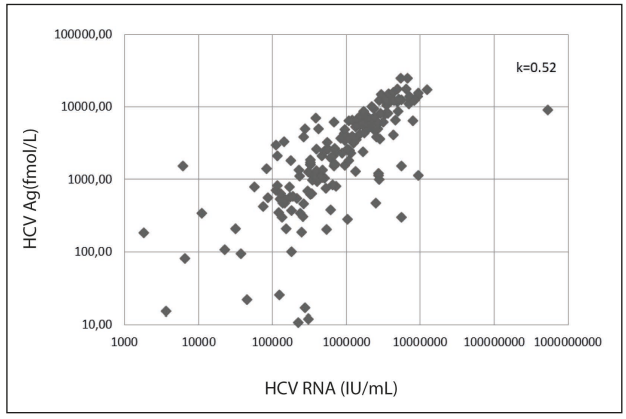
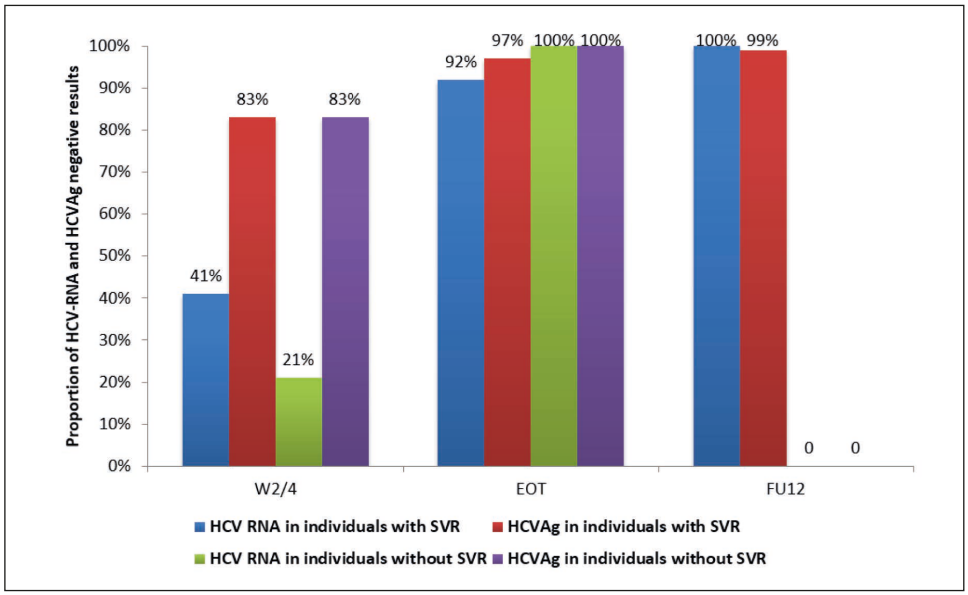


Figure 2 - *Correlation between HCV-RNA and HCVAg during and after treatment.*

Figure 3 - *Frequency of negative results for HCV-RNA and HCV-Ag during on-treatment and follow-up monitoring of 180 patients treated with DAAs for chronic HCV-infection according to sustained virological response.*



Undetectable HCV-RNA at EOT showed sensitivity 54%, specificity 100%, negative predictive value (NPV) 93% and positive predictive value (PPV) 100%. Concerning HCVAg, the negativity rates were 83% and 83% at W2/4, 97% and 100% at the end of treatment, respectively (*Figure 3*). Undetectable HCVAg at EOT showed sensitivity 74%, specificity 100%, NPV 97% and PPV 100%.

DISCUSSION

Current DAAs regimens are generally well tolerated and expected SVR is >95% after a relatively short-term treatment. Monitoring of treatment efficacy is based on measurements of HCV-RNA levels in serum or plasma, ideally at the same laboratory for each individual at different time points, including baseline and 12 or 24 weeks after the end of therapy, to verify SVR12 or SVR24, respectively.

According to several reports, confirmation of the ability of HCVAg as a marker of viral replication comparable to HCV-RNA (Aghemo A., 2016; Loggi E., 2017; Chevaliez S., 2018), measurements of HCVAg levels in serum or plasma by means of chemiluminescent microparticle immunoassay (CMIA) is indicated as an alternative to HCV RNA level measurements in settings where HCV RNA assays are not available and/or not affordable (EASL, 2020; WHO, 2018).

Most recent World Health Organization testing guidelines indicate that a lower level of analytical sensitivity can be considered if an assay is able to improve chronic HCV infection test access and/or treatment monitoring affordability. An assay with a limit of detection of 3,000 IU/mL or lower would be acceptable and would identify 95% of those with viremic infection, based on the available data (WHO, 2018). This level is equivalent to the good correlation of the HCVAg assay with HCV RNA above 3,000 IU/mL (WHO, 2018).

In the present study the correlation between HCVAg and HCV-RNA at baseline and during monitoring was fair (Spearman; r=0.80 and 0.75, respectively) with moderate qualitative agreement overall (kappa=0.52). Both features are lower than reported in previous studies (Aghemo A, 2016; Loggi E, 2017; Chevaliez S, 2018) and this is due mostly to discordant results and the lowest level of correlation found during the first weeks after treatment initiation, as reported in previously published research (Loggi E, 2017). The different kinetics of RNA and Ag and the population increase, including only interferon-free regimens, compared to the previous study (Loggi E., 2017), could be taken into account to explain discrepancies.

Interestingly, the study showed that with cut-off points at 10 fmol/L, considering a gray zone in the interval between 3 and 10 (as suggested by the manufacturer), HCVAg assesses 100% (180/180) of active viremic HCV infections at baseline and 100% (14/14) of DAAs failures during follow up after completing treatment. There were only 2 discrepant cases regarding the assessment of SVR at 12 weeks post-treatment and in both the HCVAg values were in the gray zone. This could suggest that follow-up costs could be reduced after DAAs treatment using HCVAg as a faster and easier assay to assess SVR even in middle- and high-income countries, saving resources to be reallocated to other health services. A known limitation of this purpose may be represented by the lower sensitivity of HCVAg in comparison with HCV-RNA, which may run the risk of not detecting viral replications with relatively low levels of RNA.

A recent review of available data from several clinical trials (Harrington PR, 2020) has demonstrated that 97% of individuals had a post-treatment Week 12 HCV RNA >10,000 IU/mL, above reported HCVAg sensitivity limits. Our data support this finding.

The main limitation of this study is the small number of DAAs post-treatment failures; its strengths are represented by the enrollment of individuals from two different clinical centers and having measured HCVAg retrospectively at the same time for each individual, avoiding laboratory variability, compared to two different RT-PCR-based methods.

Testing for HCVAg has already demonstrated very high specificity and positive predictive value as a marker for diagnosis of active HCV infection (EASL, 2020; WHO; 2018; Chevaliez S., 2018) and offers economic advantages for HCV population screening and treatment monitoring (Popping S., 2019).

The findings of the present study suggest the high accuracy of HCVAg measurements if compared to HCV-RNA in a real-life setting in chronic HCV diagnosis, DAAs treatment monitoring, and assessment of therapeutic failures in HCV infection.

CONCLUSION

Our data support the indication for HCVAg testing in DAAs treatment monitoring. According to the initial virological response in all individuals, the high sensitivity and negative predictive value, and given the operative and economic advantages of the HCVAg, we suggest a monitoring algorithm for DAAs treatment with HCV-RNA tested at baseline and either HCV-RNA or HCVAg at the end of treatment, since the assays provide similar results, while HCVAg may be employed as a stand-alone assay to verify the SVR, especially in low-resource settings but even in other settings to contribute to optimized resource allocation.

Conflicts of interest

The laboratory reagents for serological determinations were provided by Abbott Diagnostics at no charge. Abbott Diagnostics partially supported the research by providing the research materials, pur-

suant to an agreement for an Investigator Initiated Study (IIS), but did not play a role in study design, data collection, decision to publish, or preparation of the manuscript. No Authors' salaries were paid.
Dr. Claudio Galli is currently employed by Abbott Diagnostics as the Global Associate Medical Director, Infectious Diseases, and owns Abbott stocks. The other Authors have no conflicting interests to declare.

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