

THE USE OF ANTI-HCV IgM AS AN ADDITIONAL
MARKER FOR HEPATITIS C VIRAL REPLICATION
IN PATIENTS WITH HEPATITIS C INFECTION

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Abstract

Hepatitis C virus (HCV) infection is diagnosed by initial testing of antibodies to hepatitis C virus (anti-HCV). Antibodies of the IgM class to HCV appear earlier than anti-HCV IgG and they persist for a few months with high titres. At the same time, anti-HCV IgM is usually found in almost all patients with acute hepatitis C and during acute exacerbation of chronic hepatitis C, which correlates to persistent viremia. Despite the fact that polymerase chain reaction (PCR) is considered as the gold standard for detection of HCV viremia, some limitations exist connected to the technical performance and costs of the test. In this study we have evaluated the detection of anti-HCV IgM antibodies as a marker of viral replication in HCV positive patients. We have detected the presence of anti-HCV IgM antibodies in all RNA positive samples. The detection of both anti-HCV and anti-HCV IgM antibodies as a first step before the testing of HCV RNA is proposed for the detection of viral replication in clinical settings.

Key words: anti-HCV IgM, viremia, diagnosis

Introduction. The prevalence of anti-HCV antibodies varies throughout Europe, from 0.12% in Belgium to 2.6% in Italy [1]. Studies of HCV infection in Bulgaria are limited. The prevalence of anti-HCV antibodies in the general population is 1.3% [2, 3]. Hepatitis C is often asymptomatic or mild in its clinical

course and no clear diagnostic criteria are available to differentiate acute cases from chronic ones. After onset of the infection, 50% to 80% of the infected individuals develop a chronic infection, up to 20% progress to liver cirrhosis and 1% to 5% – to primary liver cancer after 20 to 30 years. The diagnosis of an acute HCV infection is based on the positive results for anti-HCV and/or HCV RNA. Detection of specific total anti-HCV antibodies by enzyme-linked immunoassay is the most widely used assay for laboratory diagnosis of HCV infections. The sensitivity of the commercial tests has increased up to 98% in the last decade, which has significantly reduced the window period [4]. Anti-HCV is detectable in 50% to 70% of the patients at the onset of clinical symptoms [5]. Anti-HCV antibodies may persist through the whole lifetime in the patients with spontaneously resolving infection [6]. Antibody response is developed against the structural, non-structural and the core viral proteins during the infection. But enzyme immunosorbent assay is ineffective to distinguish viremic from non-viremic patients. It has been established that approximately 85% of the people with acute infection develop persistent viremia [7]. Measurement of the HCV RNA in the sera is based on the use of nucleic acid amplification technology (NAT)-based tests, such as PCR and transcription-mediated amplification. Despite the fact that HCV RNA detection test is considered as the gold standard for detection of viremia, there are some disadvantages in it: 1) RNA present in the sample is unstable and the sera must be tested as soon as possible; 2) there is a risk of contamination; 3) the method requires specific equipment and expensive reagents, which has a significant impact on the final cost of each test.

The presence of IgM antibodies against hepatitis B virus correlates with ongoing viral replication, liver disease activity and response to antiviral treatment [8]. Antibodies from class IgM to hepatitis C virus have been found in both acute cases and in patients with exacerbation of chronic hepatitis C, where they are used as a marker for active viral replication in immunocompetent patients. It is proposed that the anti-HCV core IgM correlates with the severity of liver disease, viral replication and response to treatment in cases of chronic hepatitis [9]. At the same time, testing for antibody avidity and anti-HCV IgM allows the diagnosis in up to 90% of the cases with acute infection [10]. The objective of the present study is to evaluate the correlation of anti-HCV IgM antibodies with viremia in patients with HCV current or past HCV infection.

Materials and methods. Patients. A total of 64 patients – 49 anti-HCV and HCV RNA positive patients, and 15 anti-HCV and HCV RNA negative persons were included in the study. The viral status of the patients was determined by detection of HCV RNA into sera samples. All 64 sera were tested for presence of anti-HCV IgM. All patients were negative for hepatitis B surface antigen (HBsAg), anti-hepatitis B core (anti-HBc) IgM and anti-hepatitis A virus (anti-HAV) IgM. Sera were collected in the National Referent Laboratory of Viral Hepatitis from 2007 to 2012.

Enzyme-linked immunosorbent assay (ELISA). Anti-HCV antibodies were determined by a commercial ELISA test NANBASE C-96. Antibodies to the following recombinant HCV antigens – core, NS3, NS4 and NS5, were detected. Anti-HCV IgM antibodies were detected by a commercial ELISA test (DIA.PRO, Italy) to the same recombinant HCV antigens – core, NS3, NS4 and NS5, with neutralization of anti-HCV IgG during the performance of the test.

Polimerase chain reaction (PCR). The quantitative determination of HCV RNA was carried out by the COBAS AmpliPrep/COBAS TaqMan HCV test (Roche Diagnostics Mannheim), with detection rate from $\geq 4.30E+01$ IU/ml to $\leq 6.90E+07$ IU/ml.

Results. 38 men (59%) and 26 women (41%) with mean age of 43 ± 18 years, ranging from 5 to 78, were included in the study cohort. The main characteristics of patients groups are summarized on Table 1. Results are reported using S/CO ratios (signal-to-cut off ratio) and anti-HCV was considered positive when the S/CO ratio was greater than 1. Anti-HCV positive samples had values ranging from 2.513 to 5.755 for the group with HCV viremia (HCV RNA positive) and from 0.603 to 5.180 for the group without viremia (HCV RNA negative).

Forty-nine (77%) of the 64 serum samples were positive for anti-HCV IgM class antibodies and forty-five (70%) for the presence of HCV RNA. All viremic sera were positive for anti-HCV IgM. Three sera positive for IgM were negative

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Main characteristics of patients groups

Patients characteristics	Mean (%)
Age	43 ± 18
Gender	
Male	38 (59%)
Female	26 (41%)
HIV positive	8 (16%)
Anti-HCV IgM positive	49 (77%)
HCV RNA positive	45 (70%)
Anti-HCV IgM + / HCV RNA +	45 (70%)
Anti-HCV IgM + / HCV RNA -	4 (6%)
Anti-HCV IgM -/HCV RNA -	15 (23%)
Total	64

for HCV RNA. Fifteen sera were negative for both markers – anti-HCV IgM and HCV RNA.

From the 8 HIV-positive patients, 7 (88%) were positive for anti-HCV IgM and 6 (75%) were positive for HCV RNA. In this group, the combination between anti-HCV IgM and HCV RNA was 6 (75%) sera – IgM-positive and RNA positive, 1 (13%) – IgM-positive and RNA negative and 1 (13%) – both IgM and RNA negative.

Discussion. Detection of specific antibodies against HCV and HCV RNA are the most widely available and used tests for diagnosis and monitoring of HCV infections. In the diagnostic settings the serological anti-HCV assay has many advantages as easy of use, low variability, easy of automation and low expense [11]. However, although anti-HCV assays are developed as highly sensitive and specific ones having passed through first to third generation, false positive results are not infrequent, especially in low-risk populations (with an anti-HCV prevalence of < 10%) [12].

Some authors have found that high level of anti-HCV antibodies in the blood can distinguish the viremic from the non-viremic samples [13]. We measured minimal anti-HCV values of 2.513 for the RNA positive samples and 0.603 for the RNA negative ones, but the maximal values were almost the same, 5.755 and 5.180 respectively. It is important to mention that depending on selection criteria the number of patients with HCV viremia among anti-HCV positive could be different [14]. In the present study, only anti-HCV positive patients are included and we have focused on the evaluation of a test easy to perform for detection of HCV viremia in clinical settings. For this reason, we measured HCV RNA and anti-HCV IgM antibodies simultaneously, which were proposed as markers for active viremia from other authors [15]. We detected the presence of anti-HCV IgM antibodies in all 45 RNA positive samples. The same correlation was observed in HIV-positive patients, all 6 sera that were positive for HCV RNA were positive and for anti-HCV IgM too. The correlation between the presence of IgM antibodies and the presence of viral RNA is reported in 76%–100% of HCV ongoing infections [16]. Our results indicate that even in the case of immunocompromised patients the predictive value of anti-HCV IgM is high as a marker of viremia. A significant increase of IgM levels is also established in the case of HCV recurrence with active viral replication in immunosuppressed liver transplant patients [17]. Detectable anti-HCV core IgM in chronic HCV infection is used as an indicator for an active immune response to persistent viral replication. Our results are in agreement with the already established correlation of 80.5% for anti-HCV core IgM antibodies and persistent viremia in chronic hepatitis C [18]. The high specificity with 90% correlation between anti-HCV IgM to different immunogenic HCV proteins and presence of HCV RNA was established by BOCHKOVA et al. [19]. COPPOLA et al. suggested anti-HCV IgM and IgG avidity index detection for patients with hepatitis C for differentiation of acute from chronic infection [20].

For all these reasons, we propose the detection of both anti-HCV and anti-HCV IgM antibodies as the first step for detection of viral replication before the testing of HCV RNA.

REFERENCES

- [1] European Centre for Disease Prevention and Control, Stockholm: ECDC, 2010.
- [2] ATANASOVA M. V., I. A. HAYDOUCHKA, S. P. ZLATEV, Y. D. STOILOVA, Y. T. ILIEV, N. G. MINERVA. *Gastroenterol. Dietol.*, **50**, 2004, No 1, 89–96.
- [3] PETRUNOV B., M. KOJOUHAROVA, P. TEOHAROV ET AL. *J. of Hepatology*, **36**, 2002, Supl. 1, 138–139.
- [4] MAJID A. M., D. R. GRETCH. *Microb. Infect.*, **4**, 2002, No 12, 1227–1236.
- [5] PAWLOTSKY J.-M. *Hepatology*, **36**, 2002, No 5, Suppl 1, S65–S73.
- [6] VILLANO S. A., D. VLAHOV, K. E. NELSON, S. COHN, D. L. THOMAS. *Hepatology*, **29**, 1999, No 3, 908–914.
- [7] DUCOS J., A. M. BIANCHI-MONDAIN, M. FRANCOIS, M. BOISSET, J. P. DRELL, F. B. BARIN, A. D. SERRE. *J. Clin. Microbiol.*, **32**, 1994, No 10, 2613–2615.
- [8] LEFRERE J. J., S. GUIRAMAND, F. LEFRERE, M. MARIOTTI, P. AUMONT, J. LERABLE, J. C. PETIT et al. *J. Infect. Dis.*, **175**, 1997, No 2, 316–322.
- [9] BIZOLLON T., S. N. SI AHMED, S. GUICHARD, P. CHEVALLIER, M. ADHAM, C. DUCERF, J. BAULIEUX, C. TREPO. *Gut*, **47**, 2000, No 5, 698–702.
- [10] SAGNELLI E., G. TONZIELLO, M. PISATURO, C. SAGNELLI, N. COPPOLA. *Antivir. Ther.*, **17**, 2012, No 7 Pt B, 1453–1458.
- [11] GRETCH D. R. *Hepatology*, **26**, 1997, No S3, 43S–47S.
- [12] MANCINI C., D. RIVANERA, D. LILLI, G. DI CUNZO, S. ANGELETTI, G. LORINO, G. M. DE SANCTIS, I. G. BARBACINI, G. LEONETTI, P. BIANCHI, L. V. CHIRCU, C. GALLI. *Clin. Diagn. Virol.*, **4**, 1995, No 4, 293–299.
- [13] DAL MOLIN G., C. TIRIBELLI, C. CAMPELLO. *Ann. Hepatol.*, **2**, 2003, No 2, 76–83.
- [14] CONTRERAS A. M., R. J. OCHOA-JIMÉNEZ, A. CELIS, C. MÉNDEZ, L. OLIVARES, C. E. REBOLLEDO, I. HERNANDEZ-LUGO, A. I. AGUIRRE-ZAVALA, R. JIMÉNEZ-MÉNDEZ, R. T. CHUNG. *Transfusion*, **50**, 2010, No 6, 1335–1343.
- [15] QUIROGA J. A., J. VAN BINSBERGEN, C. Y. WANG, M. PARDO, S. NAVAS, C. TRINES, M. HERRERO, V. CARREÑO. *Hepatology*, **22**, 1995, No 6, 1635–1640.
- [16] SEO Y. S., E. S. JUNG, J. H. KIM, Y. K. JUNG, J. H. KIM, H. AN, H. J. YIM, J. E. YEON, K. S. BYUN, C. D. KIM, H. S. RYU, S. H. UM. *KOREAN. J. Intern. Med.*, **24**, 2009, No 4, 302–308.
- [17] CASINO C., D. LILLI, D. RIVERA, A. COMANDUCCI, M. ROSSI, G. CASCIARO, I. PECORELLA, D. ALFANI, C. MANCINI. *J. Clin. Microbiol.*, **37**, 1999, No 8, 2726–2728.
- [18] MARTINELLI A. L., D. BROWN, H. B. BRAUN, G. MICHEL, G. M. DUSHEIKO. *J. Hepatol.*, **24**, 1996, No 1, 21–6.

- [¹⁹] BOCHKOVA G., S. FOMINA, V. PUZYREV, A. OBRIADINA, A. BURKOV, T. ULANOVA. Viral Hepatitis Congress, 7–9 September, 2012, Frankfurt, Germany. PO2.
- [²⁰] COPPOLA N., R. PISAPIA, G. TONZIELLO, A. MASIELLO, S. MARTINI, M. PISATURO, V. MESSINA, C. SAGNELLI, M. MACERA, G. SIGNORIELLO, E. SAGNELLI. J. Clin. Virol., **46**, 2009, No 3, 222–229.

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