CLINICAL IMPORTANCE OF ASSESSMENT OF ANTI-HCV IGM ANTIBODIES IN CHRONIC HEPATITIS C

J. STRÁNSKÝ[1], E. HONZÁKOVÁ[2], J. VANDASOVÁ[2], M. HOŘEJŠOVÁ[3], J. KYNČL[4], V. NĚMEČEK[5], J. HORÁK[1]

[1]First Internal Clinic of Third Medical Faculty of Charles University, Šrobárova 50, 100 34 Prague; [2]Virological Department of Regional Hygiene Station, Faculty Hospital Bulovka, Prague; [3]Second Internal Clinic of Third Medical Faculty of Charles University, Prague; [4]Third Medical Faculty of Charles University, Prague; [5]National Institute of Health, Prague, Czech Republic

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Summary. – In the majority of patients with acute hepatitis C the anti-HCV IgM antibodies in serum were present, however, some patients with chronic hepatitis C were positive for anti-HCV IgM too. The aim of this study was to assess the presence of anti-c22 IgM in patients with chronic hepatitis C and to determine whether the positivity for anti-c22 IgM has an impact on the histological finding in the liver. A total of 88 patients were examined (44 women, 44 men), mean age 48 years. The first group comprised 24 patients positive for both anti-HCV IgG and anti-c22 IgM, the second group 38 patients positive for anti-HCV IgG only, and the third group 26 patients negative for both anti-HCV IgG and anti-c22 IgM. Of 62 anti-HCV-IgG-positive subjects 24 (39%) were positive also for anti-c22 IgM. Of 24 patients who received a blood transfusion 9 (37.5%) were positive for anti-c22 IgM. The mean serum alanine aminotransferase (ALT) activity was significantly higher in subjects with anti-c22 IgM than that in subjects without them ($p = 0.006$), however, the difference in aspartate aminotransferase (AST) was not significant ($p = 0.09$). Histological examination was performed in 46 patients. Two-thirds of the patients with anti-c22 IgM had either cirrhosis or chronic active hepatitis (CAH) while only one third of the anti-HCV-positive patients without anti-c22 IgM had CAH or cirrhosis. The results showed that approximately 40% of the patients with CAH and cirrhosis had anti-c22 IgM, a significantly higher serum ALT activity and more serious histological finding in the liver than anti-HCV-positive patients without anti-c22 IgM.

Key words: chronic hepatitis C; anti-c22 immunoglobulin M

Introduction

HCV is the main cause of parenteral non-A, non-B hepatitis. HCV infection is usually diagnosed by assays which detect circulating antibodies to HCV antigens but these only reflect the immune response and do not indicate active viraemia (Lau *et al.*, 1993). At present, second generation ELISA methods are used, however, they cannot distinguish between acute and chronic HCV infections. It was reported recently that they can be distinguished by means of IgA antibodies generated against HCV core antigen (Sato *et al.*, 1994).

In acute hepatitis C early anti-HCV IgM antibodies can be detected in serum already between the 1st and 4th week; its presence correlates with the peak of ALT activity and persists on average for 9 weeks. Anti-HCV IgM persist after acute infection in patients who contract chronic hepatitis C (Quiroga *et al.*, 1991). However, positive anti-HCV IgM were detected also in patients with chronic hepatitis C where the presence of anti-HCV IgM correlate with active viral replication, elevated serum transaminase activity and active liver disease (Brillanti *et al.*, 1992; Yuki *et al.*, 1995). Disease remission is usually associated with significantly decreased levels of anti-HCV IgM (Mergener

Abbreviations: A = absorbance; CAH = chronic active hepatitis; HCV = hepatitis C virus; IFN = interferon; iv = intravenous

et al., 1992). The assessment of anti-HCV IgM provides a useful diagnostic tool to distinguish patients with chronic hepatitis C from asymptomatic HCV carriers (Capalgo *et al.*, 1993) and for antiviral treatment.

In the Czech Republic so far no data were published in the prevalence of anti-HCV IgM in patients with chronic hepatitis C.

The aim of this study was to assess the presence of anti-HCV IgM in patients with previously confirmed chronic hepatitis C, in subjects with serious liver damage in particular CAH and HBsAg-negative cirrhosis of the liver, with elevated serum transaminase activity, and with a case-history of some risk factor of HCV infection in particular blood transfusion, and in excluded blood donors. We compared the histological findings in anti-HCV IgM-positive and negative patients.

Materials and Methods

Patients. In 1993, a total of 88 patients (44 women, 44 men) were examined. Forty-five patients suffered from chronic liver disease usually confirmed by histological examination (CAH, cirrhosis, chronic persisting hepatitis), 23 were blood donors excluded from blood donation because of confirmed anti-HCV-positivity or elevated transaminase activity, 18 had other liver diseases (fibrosis, steatosis, non-specific changes), and 2 had resolving acute hepatitis C. The patients included one intravenous (iv) drug addict, one man with extensive tattooing but none treated with interferon. Forty-six patients were histologically examined by blind liver biopsy, the remaining 42 subjects including 23 blood donors were not.

With regard to the serological examination of anti-HCV IgG and IgM antibodies the patients were divided into three groups: group 1 – patients positive for both anti-HCV IgG and IgM; group 2 – patients positive for anti-HCV IgG and negative for anti-HCV IgM; group 3 – patients negative for both anti-HCV IgG and IgM.

Anti-HCV IgG and IgM antibodies. The serum specimens stored at -20°C were assayed in the course of 3 months for the anti-HCV IgG antibodies by the Monolisa anti-HCV Sanofi Pasteur test, and for the anti-HCV IgM antibodies with the Abbott IgM HCV EIA kit according to the instructions of the manufacturers. The Abbott kit specifically detects IgM antibodies directed against the putative HCV core protein as described by Kapprell *et al.* (1993).

Serum transaminases, ALT and AST, were assayed in all patients by standard procedures.

Statistical evaluation of the differences between groups was done by the paired t-test.

Results

The basic data on the number of patients in the groups, sex, male/female ratio, mean age and mean serum ALT and AST activities are presented in Table 1.

Table 1. Basic data on the examined patients

Groups	Patients					Mean activity (μkat/l)	
	Total	Men	Women	M/F ratio	Mean age (range)	ALT	AST
1	24	14	10	1.4:1	55 (23–81)	1.98 (0.52–4.31)	1.43 (0.48–3.21)
2	38	15	23	1:1.53	46 (12–73)	1.30 (0.20–4.47)	1.08 (0.35–3.74)
3	26	15	11	1.36:1	43 (19–75)	1.01 (0.26–5.11)	0.81 (0.27–2.36)
Total	88	44	44	1:1	48 (12–81)	1.43 (0.20–5.11)	1.11 (0.27–3.74)

Normal values for both ALT and AST were lower than 0.70 μkat/l.

Of 88 examined subjects 62 were positive for anti-HCV IgG (70%), the remaining 26 were negative. Of 62 anti-HCV IgG-positive patients 24 (39%) were positive also for anti-HCV IgM, and the same number (24) had a blood transfusion in their history but these were not the same patients. Nine of 24 patients (37.5%) were positive for anti-HCV IgM and had a history of blood transfusion. In the whole group, there was a balanced male/female ratio (1:1). The age of difference between groups 1 and 3 was due to the younger age of blood donors, of whom 14 out of the total number of 23 were in group 3.

The mean absorbance of anti-HCV IgG in group 1 was 2.452 (range 0.660 – above 3.000). The quantitative distribution of the absorbance in different patients is given in Table 2.

Table 2. Absorbance of anti-HCV IgG in group 1 patients

Patients	Number	A
Strongly IgG-positive	20	2.000–above 3.000
Slightly IgG-positive	3	0.660–1.999
IgG-negative 1	0.297	

It demonstrates that the majority of patients were strongly positive for anti-HCV IgG. One women with liver cirrhosis and portal hypertension had repeatedly negative anti-HCV IgG but since anti-HCV IgM were unequivocally and repeatedly positive she was included in group 1.

The mean absorbance of anti-HCV IgM was 0.920 (range 0.166 – above 2.000). The quantitative distribution of the absorbance in individual patients is presented in Table 3. It shows that the majority of patients was strongly or medium anti-HCV IgM-positive. One patient with a very slight IgM

anti-HCV IgM-positive. One patient with a very slight IgM positivity had a receding acute HCV infection and the check-up examination of IgM one month later was already negative. Two months later he was negative also for anti-HCV IgG, however, in the course of 1994, he was again twice positive for anti-HCV IgG, and in 1995 he developed chronic carrier state while the transaminases were quite normal.

Table 3. Absorbance of anti-HCV IgM in group 1 patients

Patients	Number	A
Strongly IgM-positive	9	1.313– above 2.000
Medium IgM-positive	9	0.276–0.916
Slightly IgM-positive	5	0.218–0.268
Very slightly IgM-positive	1	0.166

However, another female patient with a medium positivity for anti-HCV IgM (A 0.323) was, as mentioned above, repeatedly negative for anti-HCV IgG. This woman was the single discrepant serological finding. Of 23 examined blood donors only one (4.3%) was slightly positive for anti-HCV IgM (A 0.218).

Group 2 comprised 38 anti-HCV IgG-positive subjects which were all anti-HCV IgM-negative. The mean absorbance of anti-HCV IgG was 2.160 (range 0.336 – above 3.000). The quantitative distribution of the absorbance in individual patients is given in Table 4 which shows that two-thirds of patients were strongly anti-HCV IgG-positive. The mean absorbance of anti-HCV IgG was higher in IgM-positive subjects than in IgM-negative ones but the difference was not statistically significant ($p = 0.104$). In group 2 there was not observed any serologically discrepant finding.

Table 4. Absorbance of anti-HCV IgG in group 2 patients

Patients	Number	A
Strongly IgG-positive	26	2.000– above 3.000
Slightly IgG-positive	12	0.336–1.999

The mean serum ALT activity was significantly higher in subjects positive for anti-HCV IgM than in negative patients as shown in Table 1 ($p = 0.006$). The mean AST activity was also higher in subjects positive for anti-HCV IgM than in negative ones, the difference, however, was not significant ($p = 0.09$). The significantly higher serum ALT activity in patients positive for anti-HCV IgM was very probably associated with active viral replication in these patients.

The histological findings in three groups of patients are summarized in Table 5. It shows that two-tirds of the patients positive for anti-HCV IgM either suffered from liver cirrhosis or CAH, while in the group of patients negative for anti-HCV IgM only one third suffered from cirrhosis or CAH. In anti-HCV negative cases only one fifth had signs of severe liver damage. These results suggested that patients with HCV infection and positive for anti-HCV IgM had a more serious finding in liver biopsy than those without IgM or HCV infection.

Table 5. Histological findings in patients' groups 1, 2 and 3

Characteristic	Groups (total No.)					
	1 (24)		2 (38)		3 (26)	
	No.	%	No.	%	No.	%
Liver cirrhosis	10		7		4	
CAH	6		5		1	
Total	16	67	12	32	5	19
Other diagnoses	4		6		3	
Not examined histologically	4		20		18	
Total	8	33	26	68	21	81

Discussion

The prognosis of chronic hepatitis C depends above all on the histological finding in the liver and proceeding replication activity of HCV. Viraemia can be detected by assessment of anti-HCV IgM or directly by assessment of HCV RNA. While the estimation of anti-HCV IgM is simple and readily available method, the examination of HCV RNA calls for a specialized laboratory and experienced staff.

In our anti-HCV positive patients anti-c22 IgM were detected in 39%. Similar results were obtained in France where in patients with chronic hepatitis C before interferon (IFN) treatment anti-HCV IgM were found in 46% in low titers (Pawlotsky *et al.*, 1994). Anti-HCV IgM were detected in patients with chronic HCV infection in 24% in Japan (Sato *et al.*, 1994) and in 51% in Spain (Quiroga *et al.*, 1991). Anti-HCV core IgM were found in 71% of patients with high HCV viraemia level in Japan (Yuki *et al.*, 1995).

After liver transplantation anti-HCV IgM were found in 52% of patients, after kidney transplantation in 25% but only in 17% of haemodialyzed patients. The majority of patients positive for anti-HCV IgM were positive also for serum HCV RNA which means that anti-c22 IgM are asso-

ciated with chronic liver disease, active viral replication and viraemia.

In our subjects with anti-HCV IgM the blood transfusion was an important risk factor — 9 of 24 patients (37.5%) received a blood transfusion. Brillanti *et al.* (1993) detected anti-HCV IgM in about 90% of patients with chronic posttransfusion hepatitis C, even several years after the onset of HCV infection. Patients who acquired HCV infection by blood transfusion had a higher viraemia than health workers and iv drug users ($p < 0.01$). These findings indicate that the mode of acquisition is an important determinant of HCV viraemia (Lau *et al.*, 1993). The only one examined iv drug addict was anti-HCV-positive (without IgM) but one man with extensive tattooing was anti-HCV-negative.

In our patients positive for anti-HCV IgM the mean ALT activity was significantly higher than that in negative subjects ($p = 0.006$) but in AST level the difference was not significant. Similar results were recorded also by other authors in acute hepatitis C and during exacerbation of chronic hepatitis C (Kapprell *et al.*, 1993). The raised ALT activity and positive anti-HCV IgM indicate active viral replication. A detection of anti-HCV IgM identifies patients with active HCV infection and HCV-related liver disease, regardless of ALT levels, and the majority of these patients are positive also for serum HCV RNA (Brillanti *et al.*, 1992).

The majority of patients in group 1 was markedly positive for both anti-HCV IgG and IgM. The lower absorbancy of the examined samples indicated a lower anti-HCV IgG concentration and thus a lower level of viral replication. This finding is consistent with the conclusions of Yuki *et al.* (1995) who proved anti-HCV IgM in particular in subjects with a high viraemia and CAH, in contrast to asymptomatic HCV carriers. However, they did not find any correlation between the presence of IgM, serum transaminase level and histological activity.

In chronic hepatitis C the high prevalence of anti-c22 IgM was found in patients with liver cirrhosis and more aggressive liver disease (Andreone *et al.*, 1992). Our results of liver histology indicate that two-thirds of patients positive for antic-22 IgM suffered either from cirrhosis or CAH. We conclude that the individuals positive for anti-c22 IgM have frequently more serious histological finding in the liver than the anti-HCV-positive patients negative for anti-c22 IgM.

The assessment of anti-HCV IgM is very important for an IFN treatment. Patients with low HCV viraemia levels are more likely to respond to IFN in a sustained fashion (Lau *et al.*, 1993). In patients with chronic hepatitis C and response to alfa-IFN treatment, the disappearance of IgM antibody to HCV predicts that the response will be sustained (Brillanti *et al.*, 1992). Patients lacking response to IFN have a significantly higher IgM concentration before the IFN treatment than those with a marked response ($p < 0.035$). The anti-HCV concentration declines significantly after the IFN treatment (Mergener *et al.*, 1992).

The study of Tassopoulos *et al.* (1994) suggests that a decline or disappearance of anti-c22 IgM may be an early predictor of successful alfa-IFN treatment of chronic hepatitis C. In contrast to the data of Tassopoulos *et al.* (1994), the study of Caporaso *et al.* (1995) suggests that the IgM response to the structural HCV core antigen cannot be considered an early predictor of successful IFN treatment. In this case, the patients suitable for IFN treatment are those with low viraemia level and without anti-c22 IgM (Lau *et al.*, 1993; Pawlotsky *et al.*, 1994). Since the polymerase chain reaction method is not available in every laboratory, the detection of anti-HCV IgM may be used as a marker of active hepatitis C regardless of the duration of HCV infection (Brillanti *et al.*, 1993).

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References

Andreone P, Attard L, Verucchi G, Cursaro C, Miniero R, Boschi A, Sprovieri G, Chiodo F (1992): Detection of IgM to HCV by RIBA in acute and chronic hepatitis C. *J. Hepatol.* **16(1)**, S56.

Brillanti S, Masci C, Ricci P, Miglioli M, Barbara L (1992): Significance of IgM antibody to hepatitis C virus in patients with chronic hepatitis. *Hepatology* **15**, 998–1001.

Brillanti S, Foli M, Perini P, Masci C, Miglioli M, Barbara L (1993): Long-term persistence of IgM antibodies to HCV in chronic hepatitis C. *J. Hepatol.* **19**, 185–187.

Capalbo M, Negro F, Saracco G, Brunetto MR, Manzini P, Calvo PL, Abate ML, Cerchier A, Oliveri F, Colombatto P, Baldi M, Piantino P, Barry Sympson EJ, Sutherland R, Bonino F (1993): Clinical significance of IgM anti-C-22 in patients with chronic HCV infection. *J. Hepatol.* **18(1)**, S33.

Caporaso N, Tuccillo C, Morisco F (1995): Basal core-IgM antibodies to hepatitis C virus are not predictive of successful alpha-interferon therapy. *J. Hepatol.* **22**, 377.

Kapprell HP, Simson B, Stiefel K, Witteler H, Klarmann R, Grimm B, Horn A, Albrecht M, Troonen H, Michel G (1993): HCV IgM EIA: Demonstration of specific detection of anti-HCV IgM core antibodies. *J. Hepatol.* **18(1)**, S30.

Lau JYN, Davis GL, Kniffen J, Qian KP, Urdea MS, Chan CS, Mizokami M, Neuwald PD, Wilber JC (1993): Significance of serum hepatitis C virus RNA levels in chronic hepatitis C. *Lancet* **341**, 1501–1504.

Mergener K, Lennartz L, Michel G, Pelzer C, Korn A, Müller R, Manns M (1992): Anti-HCV IgM antibodies in chronic

hepatitis C as predictors of the clinical outcome of interferon therapy. *J. Hepatol.* **16(1)**, S50.

Pawlotsky JM, Darthuy F, Rémiré J, Roudot-Thoraval F, Udin L, Babany G, Duval J, Dhumeaux D (1994): The presence of anti-HCV core IgM antibodies before treatment predicts the response to interferon therapy in chronic hepatitis C. *J. Hepatol.* **21(1)**, S121.

Quiroga JA, Campillo ML, Castillo I, Bartolomé J, Porres JC, Carreno V (1991): IgM antibody to hepatitis C virus in acute and chronic hepatitis C. *Hepatology* **14**, 38–43.

Sato S, Fujiyama S, Tanaka M, Goto M, Taura Y, Kawano SI, Sato T, Yasuo H (1994): IgM and IgA antibodies generated against hepatitis C virus core antigen in patients with acute and chronic HCV infection. *Dig. Dis. Sci.* **39**, 2022–2031.

Tassopoulos NC, Hatzakis AE, Papatheodoridis GV, Karvountzis G, Troonen H, Lennartz L (1994): Early prediction of successful alpha-interferon therapy of chronic hepatitis C by core-IgM antibodies to hepatitis C virus. *J. Hepatol.* **20**, 305.

Yuki N, Hayashi N, Ohkawa K, Hagiwara H, Oshita M, Katayama K, Sasaki Y, Kasahara A, Fusamoto H, Kamada T (1995): The significance of immunoglobulin M antibody response to hepatitis C virus core protein in patients with chronic hepatitis C. *Hepatology* **22**, 402–406.