IgM antibody against hepatitis B core antigen (IgM anti-HBc): diagnostic and prognostic significance in acute HBsAg positive hepatitis

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Abstract

IgM antibody against hepatitis B core antigen (IgM anti-HBc), a marker of recent hepatitis B virus infection, was sought by radioimmunoassay in sera diluted 1/4000 from 376 patients presenting to four centres in Italy with acute, apparently type B hepatitis (hepatitis B surface antigen (HBsAg) positive).

In 320 patients (85%) a positive IgM anti-HBc test result confirmed that hepatitis was due to primary infection with hepatitis B virus. In the remaining 56 patients absence of the IgM marker indicated that they were previously unrecognised long term carriers of HBsAg. Further serum analysis often showed delta infection and occasionally hepatitis A or cytomegalovirus infection as the true cause of their illness. After six to eight months circulating HBsAg persisted in 38 of 45 patients (84%) without IgM anti-HBc but in only six of 150 patients (4%) with the IgM antibody ($p < 0.0001$).

A negative IgM anti-HBc test result in patients with acute HBsAg positive hepatitis points to a factor other than hepatitis B virus as the cause of the liver damage and predicts the carriage of HBsAg.

Introduction

Testing with sensitive immunoassays for IgM antibody against hepatitis B core antigen (IgM anti-HBc) shows that reactivity is invariably present in acute hepatitis B but usually absent or detectable only at low titres in asymptomatic carriers of hepatitis B surface antigen (HBsAg).¹⁻⁸ Longitudinal studies confirm that IgM anti-HBc is increased early during primary hepatitis B virus infection and then declines in a few months regardless of the persistence of circulating HBsAg.^{4,7}

In view of the temporal relation to recent hepatitis B virus infection measurement of the IgM antibody might help to distinguish true hepatitis B from non-B hepatitis occurring in previously unrecognised carriers of HBsAg; many of these patients presenting with an acute liver illness are likely to be diagnosed as hepatitis B on finding HBsAg in the serum.

To determine the clinical value of IgM anti-HBc, this serum marker was measured by radioimmunoassay in 376 patients with

acute, apparently type B hepatitis (HBsAg positive) presenting to centres in Italy serving populations with various prevalences of carriage of HBsAg. The sensitivity of the test in discriminating recent from old infection with hepatitis B virus was optimised using sera from patients with true acute hepatitis B and from asymptomatic long term carriers of HBsAg.

Materials and methods

DETECTION OF IgM ANTI-HBc

IgM anti-HBc was measured with a μ capture radioimmunoassay, whose specificity has been described.⁴ The test was performed on beads coated with antihuman IgM (AU-IGMK, Sorin Biomedica, Saluggia, Italy) diluted 1/25 000 in phosphate buffered saline 150 mmol/l, pH 7.4. Coated beads were incubated overnight at room temperature with 200 μ l test serum diluted in phosphate buffered saline containing 4% bovine serum albumin and 1 mmol edetic acid (EDTA)/l (29.2 mg/100 ml). After washing, beads were incubated for three hours at 45°C with 50 μ l standard hepatitis B core antigen (HBcAg) plus 150 μ l ¹²⁵I labelled IgG anti-HBc (about 3×10^5 counts/min/well); 10 μ l of a 3% solution of Nonidet P40 was added to each bead. After a final washing in deionised water the beads were counted for residual radioactivity. Sera were considered positive for IgM anti-HBc if the number of counts/min recovered from beads incubated with test serum was 2.1 times the average number of counts in negative controls. Titres of the antibody were expressed as the end point positive dilution.

HBcAg was extracted from postmortem liver of a patient who had been receiving dialysis, which displayed strong HBcAg staining on immunofluorescence; serum from the patient contained a low titre of anti-HBc. Blocks of liver were cut, washed in cold phosphate buffered saline, resuspended in TKM buffer (trometamol (TRIS) 5 mmol/l (60.6 mg/100 ml), KCl 25 mmol/l (186.4 mg/100 ml), MgCl₂ 2 mmol/l (19.0 mg/100 ml), pH 7.4), homogenised, and sonicated for 30 seconds. The homogenate was left stirring overnight at 4°C and then centrifuged for 30 minutes at 19 000 rpm with a fixed angle type 35 Beckman rotor. The supernatant was titred with a microtitre radioimmunoassay specific for HBcAg⁹ and divided into aliquots; the appropriate dilution was used as standard HBcAg for routine testing.

IgG for labelling with ¹²⁵I was obtained by DE 52 chromatography from the serum of a chronic carrier of HBsAg with a high titre of anti-HBc and negative for antibody against HBcAg.

SERUM CONTROLS

Sera used to establish the optimal sensitivity of the IgM anti-HBc assay in discriminating recent from old hepatitis B virus infection were obtained from 30 patients with true acute type B hepatitis and from 30 asymptomatic patients with chronic hepatitis B virus infection of long standing.

Patients with acute hepatitis type B were medical or laboratory staff known to have been seronegative for HBsAg before the episode of acute hepatitis accompanied by the appearance of HBsAg in serum; in each case the illness resolved uneventfully with seroconversion to anti-HBs. Serial samples of serum taken during the hepatic and convalescent phases up to 7-10 weeks from the onset of the disease were available from all.

Patients with asymptomatic chronic hepatitis B virus infection had been known carriers of HBsAg for two to five years before blood sampling; all had normal liver histologically, 15 had HBcAg in the serum, and 15 had antibody against HBcAg.

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OTHER ASSAYS

HBsAg and antibody (anti-HBs), HBeAg and antibody (anti-HBe), and IgM antibody against hepatitis A virus (IgM anti-HA) were tested with commercial radioimmunoassays (AUSRIA II, AUSAB, KIT HBe, HAVAB-M; Abbott Laboratories, Chicago). IgM antibody against cytomegalovirus (IgM anti-CMV) was measured by indirect immunofluorescence (Electro Nucleonics Laboratories Inc, Columbia, Md). Delta antigen (δ Ag) and antibody of IgG type (anti- δ) and IgM type (IgM anti- δ) were measured by radioimmunoassay.^{10 11}

PATIENTS

IgM anti-HBc was sought in a total of 376 patients with apparently type B hepatitis who had presented consecutively over one to three years to medical centres in Italy. Centres were chosen from areas with different rates of carriage of HBsAg in the populations. There were 89 patients from Torino (Piedmont), 85 from Lodi (Lombardy), 90 from Siena (Tuscany), and 112 from Napoli (Campania); rates of carriage of HBsAg in these areas were reported as 1.7%, 6.2%, 1.2%, and 4.3%.^{12 13}

The diagnosis was based on presentation with symptoms suggestive of acute viral hepatitis and an alanine aminotransferase activity 10 times or more the normal value, a normal serum protein concentration, and HBsAg in serum. None of the patients had evidence of previous liver disease; the acute illness was fatal in one patient. Serum was obtained within one week after admission to hospital. A follow up serum sample was obtained from some of the patients six to eight months after the onset of hepatitis and examined for HBsAg and alanine aminotransferase activity.

Percentage prevalence of HBsAg carriers and rate of IgM anti-HBc negative hepatitis B in four medical centres in Italy.

Results

CONTROL SERA

Sera from controls with true acute hepatitis B and with chronic asymptomatic hepatitis B virus infection were titred for IgM anti-HBc in 10-fold dilutions. Controls with hepatitis B were all positive at a titre of $1/10^5$ or higher in the acute phase of the illness, decreasing to not less than $1/10^4$ within two months after onset. Long term carriers of HBsAg were all negative at a dilution higher than $1/5 \times 10^2$. A $1/4000$ dilution of serum was therefore used to screen for IgM anti-HBc. Positive sera were titred to end point.

IGM ANTI-HBc IN ACUTE HEPATITIS

Of the 376 patients with acute, apparently type B hepatitis, 320 (85%) were positive for IgM anti-HBc at titres between $1/10^5$ and $1/10^6$ and 56 (15%) were negative at a serum dilution of $1/4000$. Of the 56 IgM anti-HBc negative patients, seven were among the 89 patients from Torino (7.8%), nine among the 85 patients from Lodi

(10.5%), eight among the 90 from Siena (8.8%), and 32 among the 112 from Napoli (28.5%).

There were 11 parenteral drug addicts and two patients receiving dialysis in the IgM anti-HBc negative group; characteristic epidemiological features were not observed in the others.

SEROLOGICAL CHARACTERISTICS OF ACUTE HBsAg POSITIVE HEPATITIS WITH AND WITHOUT IGM ANTI-HBc

Sera of 239 patients with IgM anti-HBc and of the 56 patients without IgM anti-HBc were examined for the HBe antigen-antibody system.

HBeAg was detected in 171 (71%) of the 239 patients with IgM anti-HBc and in 15 (26%) of the 56 patients without the IgM marker. Anti-HBe was detected in 60 (25%) of the 239 patients with IgM anti-HBc and in 38 (68%) of the 56 without the IgM marker. HBeAg was significantly associated with the presence of IgM anti-HBc ($p < 0.001$; 95% confidence limit of the difference: 31.8-57.7); anti-HBe was significantly associated with the absence of IgM anti-HBc ($p < 0.001$; 95% confidence limit of the difference: 29.3-56.2).

IgM anti-HBc negative sera were further screened for IgM anti-HA, IgM anti-CMV, and markers of delta infection. One patient (1.7%) had IgM anti-CMV, three (5.3%) had IgM anti-HA, and 32 (57%) had markers of delta infection. Delta markers were: δ Ag in one patient, IgM anti- δ in four patients, and IgM plus IgG anti- δ in 27 patients.

Follow up serum samples were obtained six to eight months after the onset of hepatitis from 45 patients without IgM anti-HBc and from 150 with the IgM marker. HBsAg was found in the serum of 38 (84%) of the 45 patients without the marker and in six (4%) of the 150 patients with the marker (table). There was a significant association between the absence of IgM anti-HBc at the onset of hepatitis and the persistence of HBsAg at follow up ($p < 0.0001$; 95% confidence limit of the difference: 69.4-91.5). Raised alanine aminotransferase activity indicated chronicity of liver disease in 31 of the 38 IgM anti-HBc negative patients with persistent HBs antigenaemia and in four of the six patients positive for IgM anti-HBc. At follow up alanine aminotransferase activity was normal in all 144 IgM anti-HBc positive patients whose hepatitis B virus infection cleared.

Serological follow up of HBsAg positive hepatitis with and without IgM anti-HBc

	No of patients followed up	Serum HBsAg after six to eight months	
		Positive	Negative
IgM anti-HBc positive	150	6*	144
IgM anti-HBc negative	45	38†	7

* Includes four with abnormal alanine aminotransferase activity.

† Includes 31 with abnormal alanine aminotransferase activity.

Discussion

In this study optimal discrimination between recent and old hepatitis B virus infection was achieved with a radioimmunoassay for IgM anti-HBc performed in sera diluted $1/4000$, at which controls with acute hepatitis B yielded invariably positive reactions, whereas chronic asymptomatic carriers of HBsAg gave consistently negative results.

In most of the patients presenting with acute HBsAg hepatitis the finding of IgM anti-HBc confirmed that the illness was due to primary infection with hepatitis B virus. In each of the series examined, however, some of the patients lacked the IgM marker and were therefore considered to be previously unrecognised carriers of HBsAg presumably suffering from non-B hepatitis. In keeping with this, on admission to hospital many of them had circulating anti-HBe, which represents a marker of inactive infection¹⁴; HBeAg was usually found in the serum of patients positive for IgM anti-HBc as typical of classical acute hepatitis B.¹⁴

Further serological analysis often disclosed delta infection and occasionally hepatitis A or cytomegalovirus infection as the true cause of the illness. An additional factor could not be identified in some of the patients; undefined non-A non-B viruses of

hepatitis or reactivation of the latent hepatitis B virus infection may have been implicated as the possible cause of the hepatic episode.

As expected, during follow up HBsAg persisted in the serum of many patients without the IgM antibody, whereas among patients with the IgM marker the antigen cleared in almost all. Clinically, therefore, a negative IgM anti-HBc test result predicts the carriage of HBsAg.

Our data show that testing for IgM anti-HBc is important in patients developing an apparent hepatitis B of unrelenting course: a similar clinical entity is mimicked by progressive non-B hepatitis in previously unrecognised carriers of HBsAg, and in this study accounted for most of the cases of HBsAg hepatitis destined to become chronic. A negative IgM anti-HBc test result should prompt search for a cause of the liver damage other than hepatitis B virus.

The magnitude of the problem of false hepatitis B is likely to be proportional to the prevalence of carriage of HBsAg in the population. In our study, however, the relation was not numerical, as in each series examined the proportion of patients negative for IgM anti-HBc was higher than the local rate of carriage.

Though a minority of the IgM anti-HBc negative patients were drug addicts or patients receiving dialysis and therefore heavily exposed to blood borne hepatitis viruses, an epidemiological factor was not obvious in the others. The implication might be that the HBsAg carrier state determines an increased biological susceptibility to hepatitis by multiple viral and possibly non-viral factors. This was shown for delta, a defective virus that is rescued and activated only by HBs antigenaemia.¹⁵

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SHORT REPORTS

Arthropathy induced by beta blockade

Few reports on arthropathy induced by beta blockade have been published. Pain and swelling of both knees developed in a man who took practolol for nearly three years after a myocardial infarction, subsided when he stopped the drug, and recurred when he took it again.¹ Polyarthritides has been described in association with propranolol and oxprenolol²; the same author described a similar case attributable to propranolol.

Paradoxically, propranolol exerts a favourable effect on arthritis.³ The mechanism of action remains to be established. The effect probably occurs as a result of the membrane stabilising or anaesthetic properties of the drug; beta receptor blockade does not seem to be a factor.⁴

In 1980 I began to look for possible cases of arthropathy induced by beta blockade after a patient's shoulder complaint subsided soon after metoprolol was stopped.

Patients, methods, and results

Patients were considered to have suffered from arthropathy induced by beta blockade if their joint symptoms developed during administration of a beta blocker and subsided only after the drug was stopped (or after a change to a different beta blocker). All the patients were seen at this hospital or my private surgery and came from a region of about 25 000 inhabitants.

Eighteen patients (mean age 64 years, range 45-74 years) were seen

with arthropathy induced by beta blockade; six had been taking beta blockers for more than five years and seven for more than two years. The primary indication was hypertension or coronary heart disease or both. In every case an attempt was made to exclude other causes of joint symptoms by radiology and serological tests for rheumatoid disease.

Fifteen patients had symptoms affecting the shoulder joint. These consisted mainly of discomfort on moving the arms and limitation of movement due to pain when the patient tried to raise the arm above the horizontal. Some patients complained of pain and stiffness at rest. None had joint effusion. Radiological examination generally disclosed slight thickening of the soft tissues surrounding the shoulder joint. Nine of the 15 had symptoms in other joints as well. Apart from the shoulder, the knee was most commonly affected (six patients). Five patients had symptoms of polyarthritides. Two patients complained of swelling and stiffness of the small finger joints.

The primary beta blocker used was metoprolol (table). Those patients who developed joint symptoms with propranolol were given metoprolol as the first alternative. All reported exacerbation of the joint symptoms. Associated symptoms such as dry mouth and eye complaints were common as well (table). Despite attempts to substitute other beta blockers for metoprolol and propranolol the same pattern of adverse effects tended to recur, so that beta blockade eventually had to be stopped in 12 of the 18 patients. All the joint symptoms (and the associated complaints) resolved promptly in the patients.

Comment

Joint disturbance must be considered to be a common adverse effect associated with beta blockade. Other adverse effects, notably eye symptoms, dry mouth, and Raynaud's phenomenon, were also common, which points to some common factor, most probably dehydration of mucous membranes and synovia induced by the drug.