Evaluation of Anti-HBc IgM Estimation by Radioimmunoassay for Anti-HBc on Column Separated IgM*

KEVIN M. DE COCK, M.D., M.R.C.P., D.T.M.&H.†
MARY ASHCAVAI, B.S.,‡
SUGANtha GOVINDARAJAN, M.D.‡
and ALLAN G. REDEKER, M.D.†

†University of Southern California Liver Unit,
‡Department of Pathology,
Rancho Los Amigos Medical Center
Downey, CA 90242

ABSTRACT

Tests for IgM antibody to hepatitis B core antigen (anti-HBc IgM) are useful diagnostic tools in the evaluation of patients with hepatitis B virus (HBV) infection. A method is described for detecting anti-HBc IgM based on application of a commercially available radioimmunoassay for total anti-HBc to column separated serum IgM and the technique is evaluated in patients with acute and chronic HBV infection. Our test is both sensitive and specific for diagnosing acute hepatitis B, although duration of positivity is highly variable. This technique is simple, inexpensive, and might be particularly useful for laboratories performing limited numbers of examinations, or with limited resources. A 45 percent savings in reagent costs is realized in our laboratory.

Introduction

Techniques for detecting IgM antibody to hepatitis B core antigen (anti-HBc IgM) are a recent and useful addition to serodiagnostic tests available for hepatitis B virus (HBV) infection.1,2,4,8,9,10,12,15,16,17,20,23 Potential uses of tests for anti-HBc IgM include the distinction of acute from chronic HBV infection,3,7,10,14 exact diagnosis of acute hepatitis B in those who rapidly clear hepatitis B surface antigen (HBsAg),11,14 and accurate diagnosis of delta hepatitis,6,19 a situation requiring distinction between coinfection with HBV and delta, and delta superinfection in an HBV carrier.

Many different techniques are currently in use for the detection of anti-HBc IgM, making comparison of results difficult. An ELISA kit is now commercially available for anti-HBc IgM detection, although relatively little published work exists describing its standardization and use in clinical practice.10 Expense may be a limiting factor to the use of such a kit by laboratories performing a limited number of examinations.
In our institution, the present authors have been assaying for anti-HBc IgM by applying a commercially available radio-immunoassay for total antibody to hepatitis B core antigen (anti-HBc)* to column separated serum IgM. An evaluation of this simple and cost-effective technique is described.

Patients, Materials and Methods

Patient Selection

Patients were drawn from the weekly Hepatitis Clinic held at the Los Angeles County-University of Southern California Medical Center. The following patients were studied:

A. Eighty patients without a history of previous hepatitis B infection who suffered acute, hepatitis B surface antigen positive icteric hepatitis, with serum bilirubin levels greater than three mg per dl and alanine aminotransferase levels above 750 iu per l. Such patients were considered clinically and for epidemiological reporting to be suffering from acute hepatitis B. The first available serum from each patient was studied.

B. Eight patients with documented chronic HBV infection (HBsAg positive for greater than six months) who presented with acute, icteric hepatitis demonstrated to be owing to hepatitis A virus (HAV) infection (anti-HAV IgM positive). Sera studied were taken at presentation with acute hepatitis A.

C. Ten patients with biochemically stable chronic HBV infection. Twenty-seven sera were selected for study, drawn at intervals from one to 27 months, with a mean interval of 8.3 months. These patients attended our clinic for long-term follow up and had no evidence of acute hepatitis.

D. Five patients with known chronic HBV infection who suffered periodic aminotransferase level elevations to above 300 iu per l. These patients were similar to those in group C; other than that, occasional enzyme fluctuations occurred. Tests for HAV IgM or delta superinfection were carried out on selected but not all cases and were negative. Seventeen sera were examined, collected at intervals of from one week to four months, with a mean interval of 2.1 months. Sera were from peaks as well as troughs of aminotransferase levels.

Follow-up

Twenty-nine patients with acute HBV infection, all but two included in A under Patient Selection, had sequential sera available for examination of persistence of anti-HBc IgM. Nineteen patients were followed through to complete extinction of anti-HBc IgM response by our technique. In the other ten, anti-HBc IgM was still detectable when the patients were lost to follow-up. The interval between sequential samples varied between patients; in most patients, however, it was of the order of two to three weeks. In patients followed through to antibody extinction, duration of antibody response was taken as the interval between presentation and the midpoint between the last positive and first negative sample. Duration of antibody response was analyzed for the different serum dilutions examined.

Methodology

Separation of IgM free of IgG from serum was achieved by using QAE-Sephadex A-50* following the method of Johnson and Libby. Briefly, four microliters of serum were added to the prepared column with a bed volume of

* CORAB, Abbott Laboratories, North Chicago, IL.

* Pharmacia Fine Chemicals, Piscataway, NJ.
one ml, after applying five ml of wash buffer (2.88 g of ethylenediamine and 4.38 g of glacial acetic acid added to deionized water to make one liter at pH 7.0). The column was then washed with 20 ml wash buffer to remove serum IgG. Following this, IgM was eluted with two ml of eluting buffer (15.65 g of glacial acetic acid, 10.6 g of sodium acetate trihydrate, and 20 g of sodium chloride added to deionized water to make one liter at pH 4.2). The IgM thus recovered has been shown to contain less than five percent IgG.13 This eluted IgM was at 1:500 dilution (four µl in 2000 µl of eluting buffer). The following modification prevented the necessity of eluting the column to dryness. Porous polyethylene discs were used at the top and the bottom of the resin bed, thus maintaining column length while washing and eluting. The eluted serum IgM was tested for the presence of anti-HBc by RIA following the procedure for total anti-HBc† at 1:500 and at 1:1500 dilutions.

Results

In table I are shown the levels of anti-HBc IgM in different groups examined. In acute hepatitis B, the sensitivity of anti-HBc IgM testing at the dilution of 1:1500 was 76.3 percent. All other patients were positive at 1:500. The specificity of anti-HBc IgM testing at 1:1500 was 100 percent with no patient in any category other than acute hepatitis B having a positive test at this dilution.

Persistence of anti-HBc IgM is shown in table II. Median and titre of antibody duration have been calculated in three groups: (1) patients with acute hepatitis B in whom anti-HBc IgM was initially positive at 1:1500, and who were followed to extinction at this dilution; (2) patients with acute hepatitis B whose anti-HBc IgM at presentation was positive at 1:1500 or 1:500, who were followed through to antibody extinction at 1:500; and (3) patients as described in (2) but who were still anti-HBc IgM positive at 1:500 when lost to follow-up. Some patients were included in more than one group.

Discussion

Many different techniques have been applied to the detection of anti-HBc IgM. They include solid phase radio- and enzyme immunoassays, as well as antibody capture assays. A simple and economic method for detecting anti-HBc IgM is described based on applying the widely available radioimmunoassay for

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† Corab, Abbott Laboratories, North Chicago, IL.
total anti-HBc to column separated IgM obtained from two different serum dilutions. At 1:1500 the test was 100 percent specific for acute hepatitis B. Although the sensitivity was only 76.3 percent at this dilution in the present study, this figure is considerably higher when patients are tested at the very beginning of illness. Some of our patients were referrals who had been ill for several weeks before seen by us.

Numbers were small for comparison, but in general few patients with chronic HBV infection had positive anti-HBc IgM tests at 1:500. The rather large proportion of patients with acute hepatitis A in chronic hepatitis B who had a positive anti-HBc IgM at 1:500 is difficult to explain. Whether this is coincidence or there is interference between the two tests needs further evaluation. Positive anti-HBc IgM tests at 1:500 are non-diagnostic. They favor acute hepatitis B in the appropriate clinical setting, but they do not exclude chronic infection.

The finding of anti-HBc IgM in some patients with chronic HBV infection is not necessarily a problem of technique but may reflect the natural behavior of this antibody response. Other workers have shown a high frequency of anti-HBc IgM antibody in chronic HBV infection, especially in patients with active disease.2,15,22 In addition, reactivation of HBV replication in chronic infection may be associated with the re-appearance of other markers of acute infection, deoxyribonucleic acid (DNA) polymerase, hepatitis B e antigen and HNB DNA.5 It may be unrealistic, therefore, for clinicians to expect an IgM antibody test to separate absolutely the acute from the chronic HBV infection.

Using our technique, anti-HBc IgM at 1:1500 was detected for a median period of four weeks following acute hepatitis B. The range of antibody duration, however, was wide ranging from 11 to 107 days. Total duration of anti-HBc IgM at 1:500, irrespective of the presentation titer, ranged from one week to 163 days, with a median of 50 days (table II). A test aiming to diagnose acute infection, undue sensitivity, and, therefore, marked prolongation of antibody positivity, could cause diagnostic confusion in our opinion. As would be expected, patients who were still anti-HBc IgM positive when last seen were lost to follow-up within the range of antibody duration described previously (table II, #3).

Despite the availability of a commercially prepared kit for anti-HBc IgM, the technique described here has widespread potential applications. Laboratories performing a limited number of examinations might save resources by buying the kit for total anti-HBc and using it for both total and IgM anti HBc. With our workload of 785 test samples last year, there was a savings of 45 percent in costs of reagents. Labor was approximately two additional hours a month. Areas where HBV infection is common and resources are scarce might particularly be benefited by this.19 This technique is used routinely in our clinical work, and it is helpful in distinguishing acute from chronic HBV infection, in evaluating delta hepatitis, in assessing re-activation of chronic hepatitis B, and in diagnosing non-B hepatitis in chronic HBsAg carriers. In addition, about 20 percent of patients with acute HBsAg negative hepatitis have been reported to be anti-HBc IgM positive, indicating the illness was acute hepatitis B with rapid clearance of HBsAg.14,11 as with all serodiagnostic tests, results must be interpreted with an understanding of the limitations of the technique and the biological behaviour of the antibody response.

References


