Cleveland Clinic Quarterly

Volume 33 October 1966 No. 4

THE HEPATITIS—INFECTIOUS MONONUCLEOSIS (HIM) TEST: A CLINICAL EVALUATION

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DETECTION of carriers of serum hepatitis among blood donors is perhaps the most serious difficulty in the blood bank program. To date it has been an unsolved problem, there being no specific test to detect a viremia or other effect of the presence of the virus. To develop such a test, the causative agent of hepatitis not only should be known, but should be available.

Infectious hepatitis and serum hepatitis are viral in origin, but information about this virus (or viruses) has been difficult to obtain, largely because of the lack of susceptible laboratory animals and suitable media for propagation of the agent in vitro. Thus, from several isolations of viral agents[1-3] associated with infectious hepatitis no practical laboratory applications have resulted. Therefore, the reports[4-8] of the isolation of a virus associated with viral hepatitis and infectious mononucleosis, of the propagation of this agent in tissue culture, and of the development of a practical serologic test for the detection of antibodies to this agent are of great importance.

In 1961, Bolin and associates[4] inoculated five human volunteers with serum from the icterogenic plasma pool number six of the National Institutes of Health. In all five of the volunteers serum hepatitis subsequently developed, and a viral agent was isolated from the blood of each

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during the course of his illness. Viruses were also isolated from the feces of patients with infectious hepatitis,[4] and from an autopsy specimen of the liver of a patient who had died from infectious mononucleosis.[6, 8] All of these viral agents were propagated in tissue culture systems of human embryonic lung. The agents were serologically similar according to complement-fixation tests and to neutralization of their cytopathogenic effects in tissue culture.[5] No cross antigenicity between these agents and 61 other known viruses could be demonstrated by these methods.[8]

A more practical method for detecting antibodies to these viruses was needed for routine use in the clinical laboratory. For this purpose latex particles were coated with the virus (SH-193) isolated from one of the five volunteers.[6] This antigen was found to be capable of demonstrating agglutinating antibodies uniformly present in the sera of patients with serum hepatitis, infectious hepatitis, and infectious mononucleosis. This serologic test is designated by the acronym HIM test.

After testing random blood sera of donors from the blood bank, it was believed that the HIM test deserved thorough clinical investigation. This report presents the results obtained to date at this institution, and a clinical evaluation of the HIM test on sera from 244 patients and 150 control subjects.

MATERIALS AND METHODS

The antigen, SH-193, used in all tests was kindly furnished by Bolin Laboratories, Inc., Glendale, Arizona. In accordance with the method described for the use of this antigen in detecting agglutinating antibodies in serum,[7] the patient's serum was tested in twofold serial dilutions beginning with a 1:5 and carried to a 1:160 dilution. One drop (0.05 ml.) of antigen was added to 0.5 ml. of each dilution of serum. The tubes were incubated at 37 C. for 30 minutes, centrifuged at 1500 r.p.m. for 15 minutes, and then read for agglutination of the latex particles. A serum control specimen using a 1:5 dilution of the patient's serum and uncoated latex particles, and an antigen control specimen using the SH-193 antigen in the diluent buffer were also used with each serum specimen tested.

SELECTION OF PATIENTS

One hundred and fifty sera specimens obtained from the routine blood specimens submitted for syphilis serologic tests comprised a control series. These specimens were obtained from the general patient population at our institution and from unselected blood donors. No attempt was made to review the medical records of these patients. One hundred specimens of sera with negative serologic tests for syphilis and 50 with one or more

reactive syphilis serologic tests were selected at random from sera submitted to the laboratory.

Furthermore, it was desirable to obtain sera of patients with various diseases, and therefore gastroenterologists and other interested clinicians were invited to order HIM tests in addition to the routine blood tests of patients with a wide variety of disorders. The sera specimens could be grouped according to three main categories of patients: those who had viral hepatitis or infectious mononucleosis (active or in the past), hepatic disease other than hepatitis, and disease not related to the liver. The sera from 244 patients in these categories were obtained and tested. Each case was listed in the appropriate category on the basis of the clinicians's final diagnosis, which was based on his clinical judgment, laboratory tests, and in 52 cases on biopsy specimens of the liver, but the diagnosis in each case was not known to the clinical pathologist until after completion of the HIM test.

All patients considered to have active serum hepatitis or infectious hepatitis presented a clinical course characteristic of these diseases, with abnormal liver function tests including an increased value for serum glutamic oxaloacetic transaminase (SGOT). Biopsies of the liver were performed in 10 of these patients, and all the diagnoses were viral hepatitis. Patients included in the group "active infectious mononucleosis" had an elevated heterophile antibody titer with an absorption pattern characteristic of this disease, and atypical lymphocytes in the peripheral blood smears.

The first category included all patients who had a diagnosis of hepatitis or infectious mononucleosis made in the past, whether confirmed or not, regardless of the diseases for which they were being examined.

In the second category, the subgroup "cirrhosis, etiology undetermined," was used for 13 patients with a type of cirrhosis for which no definite cause could be established. Biopsies of the liver were done in 10 of these patients; the diagnosis of cirrhosis was confirmed, but no definite cause was detectable from the morphologic pattern.

All patients with obstructive jaundice had the diagnosis proved by operation, biopsy, or autopsy.

No evidence of hepatic disease was present in the 71 patients listed in the third category—disease not related to the liver. Biopsy specimens of the liver were available for 20 of these patients. The results were either entirely normal, or showed only slight, unimportant morphologic changes. A wide variety of diseases of various causes was represented in the group "systemic disease." Since the four cases of chronic ulcerative colitis constituted the largest number of cases of any one single disease, we did not feel justified in subdividing this group into disease entities.

Finally, for 11 jaundiced patients no definite diagnosis was established, and the cases were excluded entirely.

RESULTS

The results of the HIM test are listed according to the three categories in *Table 1*. Of the 150 control specimens, 100 were negative in the syphilis serologic tests, and 45 percent of these specimens had positive HIM tests; of the 50 specimens with reactive syphilis serologic tests, 38 percent were positive with the HIM antigen. In these two control subgroups the incidences can be combined (since the reactive serologic test for syphilis appears to have no effect on the HIM test) for a 42 percent incidence of positive HIM tests in the control group. Ten of 12 patients with infectious hepatitis and 12 of 14 with serum hepatitis had positive HIM tests. Of 27 of those patients with a history of hepatitis sometime in the past, 22 had positive HIM tests; for the five patients with negative HIM tests, the histories for hepatitis were not well documented. All 22 patients with the diagnosis of infectious mononucleosis as well as six of the seven with a history of infectious mononucleosis had positive HIM tests.

Of 69 patients with hepatic disease other than hepatitis, 30 (43 percent) had positive HIM tests. Even the subcategories of this group show that sera representing all diseases of the liver which were tested, with the exception of hepatitis, have incidences of positive HIM tests similar to those of the control sera.

In the last category, diseases not related to the liver, 32 (43 percent) had positive HIM tests, which again is in close agreement with the results in the control group. The group "elevated heterophile titer, not mononucleosis" were patients whose heterophile antibody titers were elevated but they did not have the differential absorption that is characteristic of mononucleosis.

The heights of the titers found in the sera of five groups of patients are illustrated in *Figure 1*. To date there has been no obvious correlation between the activity of viral hepatitis and the height of the titer. However, the titers were higher in the group of patients with active infectious mononucleosis than in the group who had viral hepatitis.

In regard to those patients with a history of hepatitis, *Table 2* shows the correlation of the height of the titer with the time interval since the year of hepatitis. No definite trend in the height of the titer is discernible from these data.

DISCUSSION

The distribution of the hepatitis virus (or viruses) is widespread. Probably a considerable number of the patients we see have had contact with the hepatitis viral agent or agents. Therefore, it is not surprising that a high percentage of the random selection of patients had agglutinating antibodies for the HIM antigen. In the control group, the 42 percent with positive

Table 1.—*Results of HIM test by categories of disease*

Category	Disease	Patients tested: Total no.	Patients tested: Positive, no. (%)
Controls	Serologic tests for syphilis, random sera		
	Reactive sera	50	19 (38)
	Negative sera	100	45 (45)
	Total	150	64 (42)
1	Hepatitis and infectious mononucleosis		
	Active infectious hepatitis	14	12 (86)
	Active serum hepatitis	16	14 (88)
	History of hepatitis	27	22 (82)
	Cirrhosis, with history of hepatitis	5	5 (100)
	Active infectious mononucleosis	22	22 (100)
	History of infectious mononucleosis	7	6 (86)
	Total	91	81 (89)
2	Hepatic disease other than hepatitis		
	Cirrhosis (no history of hepatitis)		
	Nutritional	12	5 (42)
	Biliary	9	4 (44)
	Etiology undetermined	15	7 (47)
	Obstructive jaundice due to		
	Carcinoma	11	4 (36)
	Stone or stricture	15	7 (47)
	Toxic hepatitis, drug induced	6	2 (33)
	Lupoid hepatitis	1	1 (100)
	Total	69	30 (43)
3	No hepatic disease		
	Functional gastrointestinal disease	23	9 (39)
	Systemic disease	34	17 (50)
	Elevated heterophile titer, not infectious mononucleosis	12	4 (33)
	Hemolytic anemia with jaundice	4	2 (50)
	Total	73	32 (43)
	Jaundice (etiology?)	11	8 (72)

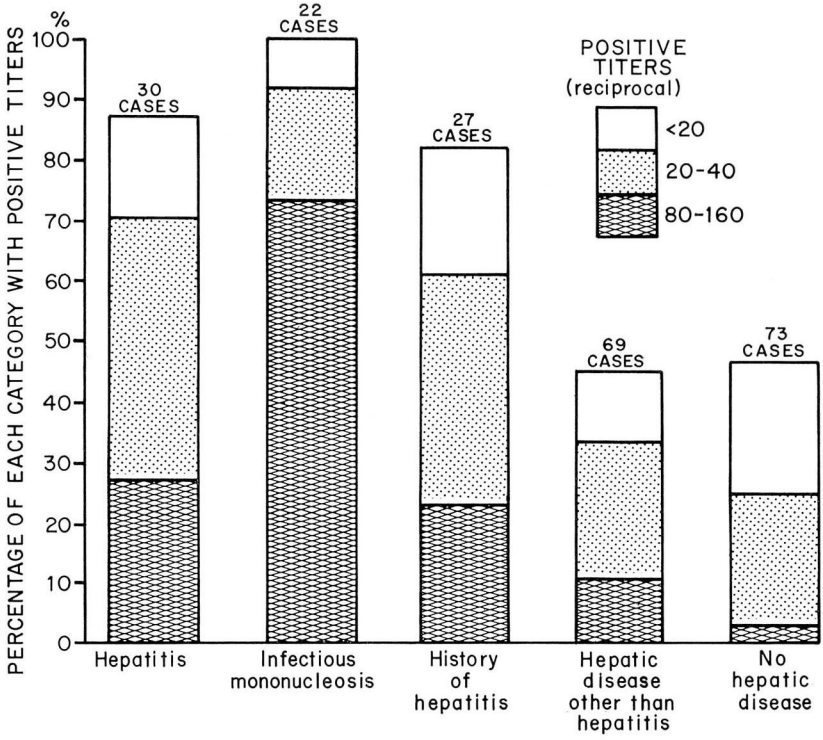

Fig. 1. Graph showing distribution of HIM titers in five groups of patients.

Table 2.—*Relationship of HIM titer to time interval since the year of hepatitis*

No. of years since the year of hepatitis	No. of patients	HIM titer (reciprocal)
1	4	10, 40, 40, 80
2	6	0, 20, 20, 40, 80, 80
3	2	0, 0
4	1	20
5	6	10, 10, 20, 20, 80, 80
6	1	0
9	2	40, 40
10	1	0
13	1	80
20	2	10, 10
30	1	10

HIM tests is consistent with the percentage found by Bolin Laboratories[7] in their screening of various population groups.

If this many random blood donors prove to have positive HIM tests, the point of practicality in rejecting this large number of donors arises. The estimated percentages of carriers of the hepatitis virus range from 0.3 to 10 percent of the entire adult population in the United States.[3] Therefore, at the present time, in using the HIM tests as the criteria for screening blood donors we would be rejecting a great many donors with antibody to the HIM antigen but with no active viremia. However, with added experience the possibility still exists that the height of the HIM titer could be indicative of the carrier state. Other facets in the use of the HIM test as a screening measure remain to be investigated, such as the possible inhibitory effect of a virus-containing serum upon a serum with an elevated HIM titer. The possibility remains, too, that perhaps the carrier rate is higher than present estimates have placed it.

The results of the clinical trial of the HIM test indicate that HIM agglutinins are present in a high percentage of the sera of patients with hepatitis and infectious mononucleosis. There were four cases of active hepatitis, two of serum hepatitis, and two cases of infectious hepatitis, in which the HIM tests were negative. One of the cases of serum hepatitis was that of a man in whom this disease developed three months after he received a renal allograft. During the postoperative period, azathioprine was included in the medication that was being administered to him. It seems possible that the action of this immunosuppressive drug could have suppressed the HIM antibody in that patient. Subsequently he received an exchange blood transfusion because of severe hepatic failure. It is interesting that the HIM titer was 1:5 at five days after the procedure, and 1:20 after 14 days.

The second patient with serum hepatitis and a negative HIM test was a man who had undergone total colectomy for chronic ulcerative colitis. Four months later jaundice developed and the diagnosis was serum hepatitis. However, this patient did take chlorpromazine intermittently for the three months before the onset of jaundice, so the possibility remains that the jaundice was actually drug induced. One jaundiced patient with chronic leukemia whose HIM test was negative was listed in the subcategory of infectious hepatitis. However, postmortem examination revealed a diffuse leukemic infiltration of the liver without evidence of viral hepatitis. We have no explanation at the present time for the negative HIM test on the other patient with hepatitis. The possibility of the presence of some inhibitory substance in the serum merits further study.

The incidence of HIM agglutinins in the sera of the patients in all other categories of hepatic disease which we investigated was similar to

that in the sera of the random population. This would indicate that disease of the liver in itself is not responsible for the presence of the HIM agglutinins. There was no apparent correlation between abnormal results of liver function tests and the presence of HIM agglutinins. In many of those patients who had hepatic disease other than hepatitis, there were notably abnormal results of liver function tests as well as negative HIM tests. Also, in many of the control subjects and in the patients who had a history of hepatitis, there were normal results of liver function tests and positive HIM tests.

In our initial experience, therefore, the HIM test seems to be a useful, new, diagnostic tool for differentiating types of jaundice. In the serum of a jaundiced patient, a negative HIM test would tend to exclude hepatitis or infectious mononucleosis as the cause of the hepatic disease.

The HIM test is not affected by elevation of the titer of the heterophile antibody other than the Paul-Bunnell antibody. Thus, the HIM test was 100 percent positive in our series of patients with infectious mononucleosis, but in those patients with heterophile antibody titers elevated from other causes, the incidence of positive HIM tests was similar to that in the control series.

SUMMARY AND CONCLUSION

The HIM (acronym for hepatitis—infectious mononucleosis) test was performed on the sera of 150 random control subjects and of 244 patients who had various diseases. The results to date indicate that the HIM antigen demonstrates agglutinins present in the sera of patients who have or had viral hepatitis or infectious mononucleosis. The HIM test appears to be of value in differentiating types of jaundice, since patients who have hepatic disease due to other causes, or who have systemic diseases, have an incidence of HIM agglutinins in their sera similar to that of the random control group. With further experience we hope for a reduction in the number of blood donors who the HIM test indicates should be rejected. The possibilities exist that the height of the HIM titer could be indicative of the carrier state, and that there would be inhibitory effects of a virus-containing serum upon a serum with an elevated HIM titer. Since it seems to offer a valuable new diagnostic tool for differentiating types of hepatic disease, a continuation of the thorough clinical evaluation of the HIM test is in progress.

REFERENCES

1. O'Malley, J. P.; Meyer, H. M., Jr., and Smadel, J. E.: Antibody in hepatitis patients against a newly isolated virus. Proc. Soc. Exper. Biol. & Med. **108:** 200–205, 1961.

2. Davis, E. V.: Reports. Isolation of viruses from children with infectious hepatitis. Science **133:** 2059–2061, 1961.

3. Taylor, A. R.; Rightsel, W. A.; Boggs, J. D., and McLean, I. W., Jr.: Tissue culture of hepatitis virus. Am. J. Med. **32**: 679–703, 1962.

4. Bolin, V. S.; Alsever, J. B.; Barger, J. B., and Jarvis, T. B.: Studies on serum and infectious hepatitis viruses of man. I. Preliminary report on the isolation of serum and infectious hepatitis viruses from man in tissue culture. Transfusion **1**: 360–366, 1961.

5. Bolin, V. S.; Brauninger, G. E.; Pardee, J., and Alsever, J. B.: Studies on the serum and the infectious hepatitis viruses of man. II. Complement fixation studies with serum and infectious hepatitis viruses. Transfusion **3**: 455–568, 1963.

6. Bolin, V. S.; Chase, B. S.; Alsever, J. B., and Mann, F. D.: Viral hepatitis test. (Abs.) Fed. Proc. **24**: 159, 1965.

7. Bolin, V. S.: Personal communication to the authors, September 15, 1964.

8. Bolin, V. S.: Personal communication to the authors, June 24, 1965.