CLEVELAND CLINIC QUARTERLY
Volume 38, July 1971
Printed in U.S.A.


# Autoimmune tests in chronic active disease of the liver

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CHRONIC disease of the liver may be defined as any progressive hepatic disease of longer than three months' duration. This definition excludes most cases of acute viral or toxic hepatitis, however there may be indicators of continuing chemical or histologic activity, such as elevation of serum transaminase or active cellular necrosis in the liver biopsy specimen. Chronic disease of the liver is a heterogeneous group of diseases with a wide range of clinical and pathologic features that tend to progress toward terminal hepatic disease with its resultant portal hypertension, ascites, and coma.

Study of the immunologic aspects of chronic disease of the liver may add to our understanding of etiology and pathogenesis, and help to evaluate better the methods of treatment. Two observations have stimulated interest in the immunologic aspects of chronic hepatic disease: (1) the presence of elevated serum $\gamma$-globulins (immunoglobulins), and (2) detection of serum autoantibodies. Since Kunkel and associates[1] found evidence of lupus erythematosus (hypergammaglobulinemia and L.E. cells) in young females with chronic liver disease, a number of circulating autoantibodies have been discovered.[2] By employing immunofluorescent technics, autoantibodies to mitochondria (AMA),[3-5] smooth muscle (SMA),[6, 7] and cell nuclei, antinuclear factor (ANF),[3, 5] have been found. Antibodies to renal glomeruli,[8] biliary canaliculi,[9] thyroid cell,[3] and gastric parietal cell[3] also have been found, but not so frequently as those in the former group. Only AMA, SMA, ANF, and L.E. tests have had wide clinical application in chronic disease of the liver.

At the Cleveland Clinic these autoantibodies and quantitative immunoglobulins have been utilized since January 1967 in the study of patients with a variety of hepatic and non-hepatic diseases. This report presents the results and the value of these tests in chronic hepatic disease.

## Materials and methods

The clinical records of all patients tested for either mitochondrial or smooth muscle antibodies at the Cleveland Clinic from January 1967 to December

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1969 were reviewed. The antibody titers and immunoglobulin concentrations were recorded. The usual indication for performing a particular test was the clinical suspicion of chronic liver disease. When serial determinations were done, the initial values were used in the tabulations.

Antinuclear factor, smooth muscle, and mitochondrial antibodies were determined by the indirect fluorescent technic, which measures antibodies present in serum. For AMA and ANF, rat kidney was the antigen, and for SMA, human uterus was used. Excised blocks of the appropriate tissue were quickly frozen and sections 4 $\mu$ thick were prepared on a cryostat. The sections were treated with patients' sera, incubated, rinsed, and overlain with fluorescent antihuman globulin (Hyland Laboratories, Los Angeles, California). Under the fluorescent microscope, the sections were examined for the characteristic apple-green fluorescence. Positive preparations were titered from dilutions of sera. Procedural details have been described previously.[10]

Immunoglobulins were determined by a gel diffusion technic using Immuno-Plate (Hyland Laboratories). The patient's serum was placed in a well in an antibody-containing agar gel plate. The immunoglobulin diffused into the agar and formed a precipitin ring, the diameter of which is related to the concentration of immunoglobulin.

## Results

A total of 146 patients had either AMA or SMA tests. Of these, 129 each had a clinical diagnosis of hepatic disease, and liver biopsy was performed in 102. AMA was tested in 115, SMA in 111, ANF in 72, and immunoglobulins in 80. There were 25 patients with non-hepatic diseases. Of 11 patients with inflammatory bowel disease, some also had liver disease.

Of the 115 AMA tests, 16 were positive (*Table 1*). Eight patients with primary biliary cirrhosis had titers of 1:160 or higher; another test was positive undiluted, but the titer was not determined. Surgical exploration was performed in six patients in conjunction with liver biopsy and operative cholangiography to substantiate the diagnosis. Another diagnosis was proved at autopsy. The patient with a titer higher than 1:160 has been followed without operative intervention. (In addition, she had an SMA titer of 1:10, and IgM of 1055 mg per 100 ml when initially seen). Three additional patients with AMA titers of 1:160 may be suspected of having primary biliary cirrhosis. Two of these patients, clinically diagnosed as having cryptogenic cirrhosis,* had ascites and varices when initially examined. Both had elevations of IgM (640 and 710 mg per 100 ml). The third patient was diagnosed clinically as having chronic active hepatitis on the basis of liver biopsy, but the SMA and ANF tests were negative; immunoglobulins were IgG 700, IgA 175, IgM 390 mg per 100 ml. All 17 patients with proved bile duct obstruction due to tumor, stricture, or stone had negative AMA tests; however in another case, previously reported,[10]

* *Cryptogenic cirrhosis is not a pathologic entity, but is a clinical designation applied to a heterogeneous group of chronic liver diseases of unproved and uncertain etiology.*

**Table 1.**—Correlation of diagnosis and test results in liver disease

| Diagnosis | Antibody test | | | | | |
|---|---|---|---|---|---|---|
| | Mitochondrial | | Smooth muscle | | Antinuclear factor | |
| | Number positive/ number tested | Percentage of total | Number positive/ number tested | Percentage of total | Number positive/ number tested | Percentage of total |
| Primary biliary cirrhosis | 9/9 | 100 | 3/6 | 50 | 1/4 | 25 |
| Chronic active (lupoid) hepatitis | 1/19 | 5 | 18/22 | 82 | 15/20 | 75 |
| Cryptogenic cirrhosis | 4/34 | 11 | 18/36 | 50 | 10/22 | 45 |
| Nutritional (alcoholic) cirrhosis | 1/6 | 16 | 2/4 | 50 | 1/2 | 50 |
| Bile-duct obstruction | 0/16 | 0 | 1/11 | 9 | 0/3 | 0 |
| Cholangiolitic hepatitis | 1/9 | 11 | 2/9 | 22 | 2/5 | 40 |
| Toxic hepatitis | 0/3 | 0 | 3/4 | 75 | 2/3 | 67 |
| Granulomatous hepatitis | 0/3 | 0 | 0/2 | 0 | 0/2 | 0 |
| Virus hepatitis | 0/13 | 0 | 8/14 | 57 | 3/7 | 43 |
| Miscellaneous liver diseases | 0/3 | 0 | 0/3 | 0 | 1/4 | 25 |
| Non-hepatic diseases | 1/25 | 4 | 4/14 | 28 | — | — |

a patient with a finely nodular cirrhosis, had an obstruction due to stone relieved surgically three years earlier, and on two occasions an AMA titer of 1:40. Among 25 control patients without evidence of liver disease, there was one positive test in a low titer (1:40).

Of 111 SMA tests performed in patients with liver disease there were 55 positive tests and the titers ranged to 1:200 (*Table 1*). Titers higher than 1:10 were considered significant. Serial testing revealed that some titers decreased or reverted to negative with time, treatment, or clinical improvement. Most of the positive SMA tests were found among the chronic active (lupoid) hepatitis (18 of 22) and cryptopgenic cirrhosis (18 of 36) groups. The higher titers were found mainly but not exclusively in these two groups. Smooth muscle antibodies were found in significant titers in eight of the ten groups of liver disease, and were present independently from ANF.

Antinuclear factor was found in titers ranging from 1:10 to higher than 1:160, in 35 of 72 patients tested (*Table 1*). Again, the majority of those titers occurred in chronic active (lupoid) hepatitis (15 of 20), and in cryptogenic cirrhosis (10 of 22).

Thirty-two patients without evidence of liver disease were tested for the presence of AMA or SMA. One patient with a renal cyst and aortic aneurysm had an AMA titer of 1:40. Significant titers of SMA occurred sporadically in myasthenia syndrome (1 patient); connective tissue disorder (2 patients), and inflammatory bowel disease (1 patient).

**Table 2.**—Autoantibodies in 11 patients with inflammatory bowel disease

| Bowel disease | Liver disease | Test | | |
|---|---|---|---|---|
| | | AMA | SMA | ANF |
| Chronic ulcerative colitis | None | Negative | Negative | Not tested |
| Chronic ulcerative colitis | None | Negative | Negative | Not tested |
| Crohn's disease | None | Not tested | Negative | Negative |
| Crohn's disease | None | Not tested | 1:10 | Not tested |
| Crohn's disease | None | Not tested | Negative | Negative |
| Crohn's disease | Chronic active hepatitis | Not tested | 1:10 | 1:20 |
| Chronic ulcerative colitis | Chronic active hepatitis | Negative | 1:50 | 1:80 |
| Chronic ulcerative colitis | Cryptogenic cirrhosis | Not tested | Negative | Negative |
| Chronic ulcerative colitis | Cryptogenic cirrhosis | Negative | Negative | Negative |
| Crohn's disease | Cryptogenic cirrhosis | Negative | 1:50 | Negative |
| Chronic ulcerative colitis | Primary biliary cirrhosis | Positive | Not tested | Not tested |

Eleven patients had chronic ulcerative colitis or Crohn's disease (*Table 2*). Of these, six had clinical liver disease: three had cryptogenic cirrhosis; two, chronic active hepatitis; and one patient had primary biliary cirrhosis. In this group, AMA was elevated in one patient (primary biliary cirrhosis) of four tested; SMA in 3 of 5 tested; and ANF in 2 of 5 tested. In the five patients without clinical evidence of liver disease, nine antibody tests were performed; the results were negative, except for one positive SMA, in a dilution of 1:10.

Quantitative immunoglobulins were determined in 80 patients. The normal values for our laboratory based on 70 determinations are listed in *Table 3*, which also shows the degrees of elevation. *Table 3* also lists the numbers of patients with normal and with elevated immunoglobulins by class, according to clinical diagnosis. Of 80 patients tested, IgG was elevated in 40, IgA in 40, and IgM in 45.

Clearly the greatest number of elevations and the highest values for IgG were found in chronic active (lupoid) hepatitis. About half of the cryptogenic cirrhosis group had elevations of IgG, but none were more than 2900 mg per 100 ml. Three of five patients with primary biliary cirrhosis showed elevations of IgG values, but these bear no statistical significance. All patients with bile-duct obstruction, granulomatous hepatitis, viral hepatitis, and miscellaneous liver disease had normal IgG values.

Elevations of IgA values were common among patients with granulomatous hepatitis and cholangiolitic hepatitis. Many of the patients with alcoholic cirrhosis, cryptogenic cirrhosis, primary biliary cirrhosis, and chronic active (lupoid) hepatitis had elevated IgA. One patient in each of the latter four groups showed IgA values greater than 950 mg per 100 ml.

The IgM value was elevated more often than that of IgG or IgA. Of five patients with primary biliary cirrhosis, four had elevations of IgM and one of these was the highest recorded in this study (1055 mg per 100 ml). Patients with cryptogenic cirrhosis had high levels and seven of these were more than 350 mg per 100 ml. Patients with chronic active hepatitis had elevations less

**Table 3.**—Immunoglobulins in liver disease

| Diagnosis | Number tested | IgG* Total elevation | IgG* I | IgG* II | IgG* III | IgG* IV | IgA* Total elevation | IgA* I | IgA* II | IgA* III | IgA* IV | IgM* Total elevation | IgM* I | IgM* II | IgM* III | IgM* IV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Primary biliary cirrhosis | 5 | 3 | 2 | — | 1 | — | 3 | 1 | 1 | — | 1 | 4 | 1 | 2 | — | 1 |
| Chronic active (lupoid) hepatitis | 20 | 16 | 5 | 1 | 6 | 4 | 8 | 3 | 3 | 1 | 1 | 9 | 5 | 3 | — | 1 |
| Cryptogenic cirrhosis | 25 | 12 | 7 | 5 | — | — | 15 | 10 | 1 | 3 | 1 | 18 | 9 | 2 | 4 | 3 |
| Nutritional (alcoholic) cirrhosis | 3 | 1 | — | 1 | — | — | 2 | — | 1 | — | 1 | 2 | — | 1 | 1 | — |
| Bile-duct obstruction | 7 | 0 | — | — | — | — | 3 | 3 | — | — | — | 2 | 1 | 1 | — | — |
| Cholangiolitic hepatitis | 6 | 2 | 1 | — | 1 | — | 5 | 3 | 1 | 1 | — | 5 | 1 | 2 | — | 2 |
| Toxic hepatitis | 3 | 3 | 2 | 1 | — | — | 1 | 1 | — | — | — | 1 | 1 | — | — | — |
| Granulomatous hepatitis | 2 | 0 | — | — | — | — | 2 | 2 | — | — | — | 1 | — | — | — | 1 |
| Viral hepatitis | 6 | 0 | — | — | — | — | 1 | 1 | — | — | — | 3 | 3 | — | — | — |

*

| Ig fraction | Upper limit of normal† (mg/100 ml) | I (mg/100 ml) | II (mg/100 ml) | III (mg/100 ml) | IV (mg/100 ml) |
|---|---|---|---|---|---|
| IgG | Up to 1900 | 1900–2400 | 2400–2900 | 2900–3400 | >3400 |
| IgA | Up to 350 | 350–550 | 550–750 | 750–950 | >950 |
| IgM | Up to 150 | 150–250 | 250–350 | 350–450 | >450 |

† Determined as mean plus 2 SD in 70 control subjects.

often. Bile-duct obstruction was infrequently associated with elevated IgM values.

## Discussion

The presence of positive autoimmune reactions in these various liver diseases suggests to us that injury to hepatic or bile-duct cells from any causative factor (drug, toxin, virus) may release protein constituents that in turn evoke the autoimmune response and perpetuate the injury to the liver. Elevated immunoglobulin values and circulating tissue autoantibodies in high titer are present in significant numbers of patients with chronic active (lupoid) hepatitis, primary biliary cirrhosis, or cryptogenic cirrhosis. A specific positive autoimmune test does not indicate that antibody itself is pathogenic, but only reflects the general autoimmune response of the patient.

The importance of the mitochondrial antibody test as a clue to the diagnosis of primary biliary cirrhosis, was previously reported.[10] It is so far the most diagnostically useful test, and detects a factor probably directed against a lipoprotein constituent of the mitochondrial inner membrane.[11] AMA occurred in titers of 1:160 or higher in eight patients definitely diagnosed as having primary biliary cirrhosis. AMA may be positive in low titer in other cholestatic forms of liver disease. It is essential to determine the titer of the AMA test; we rarely have seen a titer higher than 1:40 except in primary biliary cirrhosis. We believe that a titer of 1:80 is highly suspicious, and a titer of 1:160 is diagnostic of primary biliary cirrhosis.

In suspected autoimmune disease, the ANF is a more valuable screening tool than the L.E. test.[12] Antinuclear factor is commonly found in many autoimmune disease processes, when searched for, and therefore ANF lacks specificity. Some authors distinguish patients with positive L.E. tests from the general group of chronic active hepatitis and label them "lupoid hepatitis." We believe that patients with chronic active hepatitis who have ANF titers higher than 1:20 may be likely subjects for immunosuppressive treatment, and therefore we find this test clinically useful.

We found antibodies to smooth muscle in titers of 1:10 or higher in the sera of patients with chronic active (lupoid) hepatitis (82 percent), primary biliary cirrhosis (50 percent), and cryptogenic cirrhosis (50 percent) as well; SMA occurred independently of AMA and ANF. It is helpful in selecting the patients with chronic active (lupoid) hepatitis who are more likely to respond to immunosuppressive treatment. Because some patients with viral hepatitis, toxic hepatitis, and alcoholic cirrhosis have significant titers of SMA, this test is less specific for chronic active (lupoid) hepatitis than AMA is for primary biliary cirrhosis.

Although immunoglobulin patterns are not diagnostic of chronic hepatic disease, several interesting relationships exist. Our study agrees in general with the observations of Feizi.[13] The three major immunoglobulin classes are usually elevated in most chronic parenchymal diseases of the liver. IgG is highest in chronic active (lupoid) hepatitis, elevated to a lesser extent in primary biliary

cirrhosis and in cryptogenic cirrhosis. IgA elevations are commonly found in alcoholic cirrhosis, but are not confined to this group. We found elevations of IgA in primary biliary cirrhosis, in cryptogenic cirrhosis and in chronic active (lupoid) hepatitis, but the higher levels did not differentiate these diagnoses. IgM reached its highest level in primary biliary cirrhosis (1055 mg per 100 ml), yet one of the five patients tested had a normal IgM value. High levels of IgM were evident in patients with cryptogenic cirrhosis, and lesser elevations were found in patients with alcoholism and bile-duct obstruction.

The discovery of the autoimmune features of some liver diseases stimulated interest in treatment with immunosuppressive drugs.[14, 15] Page, Good, and Pollara[16] use the laboratory criteria of hypergammaglobulinemia ($\gamma$-globulin $>5$ g per 100 ml), and a positive L.E. test to predict the patients who are likely to respond to such treatment. We believe that patients with chronic active hepatitis who have positive autoimmune tests may be subjects for immunosuppressive treatment, which should be started early in the course of the illness before irreversible cirrhosis and its sequelae have developed.[17] Positive SMA and ANF in the active phase of the disease may subside in titer or revert to negative during such treatment, but the significance of this phenomenon is not known.

In our experience, patients with primary biliary cirrhosis have not responded to immunosuppressive treatment as well as patients with chronic active (lupoid) hepatitis. Antibody levels remain constant and are not related to the stage of the disease or changed by the treatment with immunosuppressive drugs. The AMA, however, does aid in differentiating primary biliary cirrhosis from bile-duct obstruction, which otherwise is not possible solely on clinical grounds. Laparotomy may be deferred in patients with AMA titers of 1:160 and greater, and concomitant elevations of IgM.

## Summary

Those diseases of the liver in which autoimmunity has been implicated and is associated with some positive autoimmune tests include chronic active (lupoid) hepatitis, cryptogenic cirrhosis, and primary biliary cirrhosis. Mitochondrial antibody (AMA) is useful diagnostically, being present in eight patients with proved primary biliary cirrhosis in a titer of 1:160, yet absent in 17 patients with proved bile-duct obstruction. Antinuclear factor (ANF) is a good screening test for autoimmune factors in liver disease and occurred in 15 of 20 chronic active (lupoid) hepatitis patients and in 10 of 22 cryptogenic cirrhosis patients. Smooth muscle antibody (SMA) appeared independently from either ANF or AMA, and was positive in 18 of 22 chronic active (lupoid) hepatitis patients, and 50 percent of those with primary biliary cirrhosis and cryptogenic cirrhosis. SMA and ANF values used together may be helpful in selecting patients with chronic active (lupoid) hepatitis for immunosuppressive treatment.

Immunoglobulin patterns are also useful in diagnosis when considered in conjunction with autoantibodies. IgA elevations were commonly found in all

three types of autoimmune hepatic disease, IgM elevations were suggestive of primary biliary cirrhosis, whereas moderate increases in IgG concentrations were most frequently associated with chronic active (lupoid) hepatitis.

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