

## AUTOANTIBODIES IN ALCOHOLIC LIVER CIRRHOSIS IN PORTUGAL\*

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Sera from 62 patients with alcoholic liver cirrhosis were tested for the presence of organ specific and non organ specific autoantibodies and compared with a healthy group as a control population. SMA was found in an overall percentage of 26% (30% among females and 23% among males). The titers were under 1/80. ANA was found in an overall percentage of 8% with low titers. The prevalence of SMA and ANA in the alcoholic group was higher than in controls ( $P < 0.001$ ;  $P < 0.05$ ). In patients with higher ethanol ingestion ( $> 200$ g/day) there was a significantly higher incidence of SMA (39% versus 13%;  $P < 0.001$ ). The higher incidence of SMA and ANA in alcoholics compared with controls, mainly in the group of higher consumption of alcohol, could be particularly helpful in the demonstration of the role of alcohol in alcoholic liver disease as a trigger or perpetuating factor of an immune reaction.

### INTRODUCTION

Alcoholic disease has become a major problem nowadays. However, there is individual susceptibility which may account for the discrepancy of pathological pictures related to alcohol ingestion.

According to Leibach (1975), the nature of the injury depends either on the intensity and duration of alcohol ingestion or on unidentified factors related to intrinsic susceptibility. Steatosis can be produced with relatively modest alcohol intake while only a small proportion of very long standing drinkers develop cirrhosis. There is epidemiological and experimental (Rubin & Lieber 1973) evidence that ethanol has a direct toxic effect on the liver. Pequignot (1961) estimated a much greater risk of developing cirrhosis among long duration drinkers with daily alcohol ingestion over 160 gr. The risk seems to be much less if the daily intake falls below 80 gr. The same author (Pequignot, 1975) lowered the risk margin to 40 g per day after a recent survey done in France. However, according to Howarth (1978) the safety margin is 70 g per day. The work done by Rubin and Lieber (1973, 1974) provided experimental evidence of a toxic effect of alcohol on baboons liver.

The possible importance of genetic factors determining susceptibility has been studied and negative results were reported by Brunt, 1971 and Lieber 1975. Nevertheless Bailey et al (1976) found a higher incidence of HLA-B8 in alcoholics who developed cirrhosis.

Sorrell and Leevy (1972) demonstrated an altered cell-mediated immunity in alcoholic hepatitis. Ethanol and acetaldehyde evoked an increase in the stimulation index of lymphocytes in patients with acute alcoholic hepatitis only when hyaline necrosis was present. Direct cytotoxicity to hepatocytes has also been demonstrated in patients with alcoholic liver disease (Cochrane et al., 1975, 1977). Also Mihás et al. (1975) suggested that cell-mediated immunity to alcoholic hyaline may perpetuate the hepatitis.

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Further support for these ideas arose from the demonstration of a lymphocyte proliferation inhibition factor (PIF) of lymphocytes from patients with alcoholic liver disease (Chen and Leevy et al., 1976) and the elaboration by lymphocytes sensitized to alcoholic hyaline of cytotoxic (Kakumu and Leevy, 1977) and fibrogenic factors (Chen and Leevy, 1973). The antigenic component of the alcoholic hyaline becomes extracellular in the early phases of alcoholic hepatitis and leads to antibody formation (Kanagasundaram et al., 1977). The glycoprotein contained in the alcoholic hyaline contains a fraction (one of the peaks on gel filtration) which is supposed to be the antigen carrier of the alcoholic hyaline (Luisada and Opper et al., 1977). Both humoral and cell-mediated immunity seem to be involved in the perpetuation of alcoholic hepatitis and contribute to the development of cirrhosis.

The aim of the present study is to screen our alcoholic patients for organ and non-organ specific autoantibodies and compare their incidence with that in a control population. We have also examined the correlation between daily alcohol consumption and the presence or absence of these autoantibodies.

## MATERIAL AND METHODS

### *Patients studied*

Sixty two patients, 27 females and 35 males (aged 31 to 68 years; mean age 55 years) were investigated. The diagnosis of alcoholic liver cirrhosis was made on clinical and biochemical grounds; in 42 cases histology was also obtained.

All patients had ethanol consumption over 100 g daily for more than 10 years.

One control group of 60 cases, chosen among doctors, laboratory staff and students was also tested. Thirty two were females and 28 were males (aged 19 to 48 years; mean age 20 years). All had alcohol ingestion under 100 g per day.

### *Immunofluorescence technique*

All sera were studied for antibodies to nuclei, smooth muscle, mitochondria, gastric parietal cell and thyroid microsomal antigens.

The method used was the indirect immunofluorescent technique, by Roitt and Doniach (1969). In all cases we used a dilution 1/10 of a polyvalent fluorescent antihuman globulin (Wellcome).

Fluorescent microscopy was carried out using a Leitz Orthoplan microscope with a Osram 200 lamp, BG 12 and BG 28 excitation filters and two barrier filters K 460 and K 470.

## RESULTS

### *Alcoholic liver cirrhosis*

Smooth muscle antibodies (SMA) were found to be present in the sera of 16 (28%) of the 62 patients. The titers were 1/40 except three cases which were 1/80. We got 30% positive females (8 out of 27) and 23% positive males (8 out of 35) (Fig. 1).

Antinuclear antibody (ANA) was found to be present in the sera of 5 (8%) of the 62 patients. The titers were all under or equal to 1/40 (Fig. 1).

Neither antimitochondrial antibody nor any of the organ-specific antibodies were found.

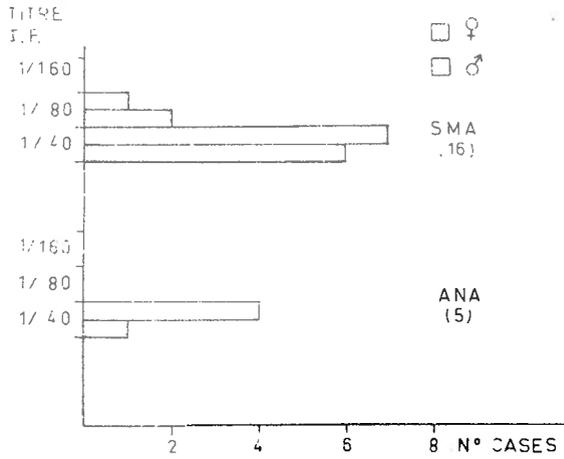


Fig. 1 — Incidence of SMA and ANA in 62 patients with alcoholic liver cirrhosis.

The patients were classified according to ethanol consumption (Fig. 2): there was one group of 31 patients with a consumption equal or under 200 g a day. In this group the SMA incidence was 13% (4 out of 31). In the group of 31 patients with daily alcohol ingestion over 200g, the SMA incidence was 39% (12 out 31).

Liver biopsy was performed in 42 patients and all showed liver cirrhosis. Of the 16 patients with positive SMA, 10 had liver biopsy. Four had superimposed alcoholic hepatitis and 3 fatty liver.

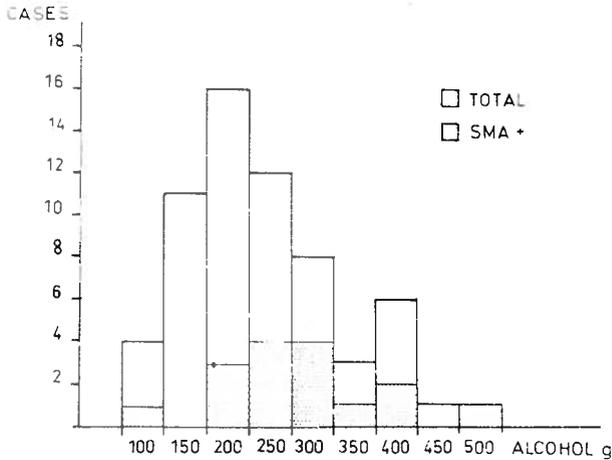


Fig. 2 — Patients distributed according to daily alcohol consumption and incidence of SMA.

*Control group*

None of the sixty patients in the control group showed SMA, ANA or any of the other antibodies. No liver biopsy was performed in this group.

## DISCUSSION

Galambos (1972) first pointed out that direct alcohol toxicity is not a sufficient mechanism for the development of liver cirrhosis, since only a small percentage of patients develop chronic disease, and this can sometimes progress even after stopping all alcohol ingestion. Alcohol may, however, play a role as a trigger of an immunological reaction, and Leevy and his group (1975) have put forward the hypothesis that hyaline bodies developed after alcohol ingestion have initiated an autoimmune process, which may later, however, progress to cirrhosis.

A circulating antigen has been isolated from alcoholic hyaline (Kanagasundaram et al., 1976), and an antibody against this antigen described. Recent work by Cochrane et al. (1977) confirms previous observations from the same group (Cochrane et al., 1975) but no correlation between the incidence of non-specific autoantibodies and cytotoxicity was found. However, Bailey et al. (1976) found an increased incidence of SMA (21%) and ANA (13%) in alcoholic patients with cirrhosis. The titres were between 1/40 and 1/80. The incidence of HLA B8 was also increased. Our results confirm Bailey's observations. 25% of our patients had SMA with titre from 1/40 to 1/80, and the ANA was positive in 8% at titres below 1/40. When compared with our controls, this is a statistically significant difference for the SMA ( $P < 0.001$ ) and ANA ( $P < 0.05$ ).

When we correlate the amount of daily alcohol ingestion and the incidence of SMA and ANA, there is a statistically significant higher incidence of SMA and ANA in the group whose alcohol consumption was over 200 g. a day ( $P < 0.001$ ).

At the present time, it would appear that alcohol could play a role not only as a trigger for the immunological reaction, but also as an important factor perpetuating an immune mechanism. From our work it seems that the presence of both SMA and ANA could be an important marker for the screening of such patients.

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