Research

Different degrees of malnutrition and immunological alterations according to the aetiology of cirrhosis: a prospective and sequential study

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Abstract

Objectives: In this work we investigated how immunological dysfunction and malnutrition interact in alcoholic and viral aetiologies of cirrhosis.

Methods: To investigate the matter, 77 cirrhotic patients divided in three aetiologies [Alcohol, HCV and Alcohol + HCV) and 32 controls were prospectivelly and sequentially studied. Parameters of humoral immunity (Components 3 and 4 of seric complement and immunoglobulins A M, G and E) and of cellular immunity (total leukocytes and lymphocytes in peripheral blood, T lymphocytes subpopulations, CD4+ and CD8+, CD4+/CD8+ ratio and intradermic tests of delayed hypersensitivity), as well as nutrititional parameters: anthropometric measures, serum albumin and transferrin were evaluated.

Results: Multiple statistical comparisons showed that IgM was higher in HCV group; IgG was significantly elevated in both HCV and Alcohol + HCV, whereas for the Alcohol group, IgE was found at higher titles. The analysis of T- lymphocytes subpopulations showed no aetiologic differences, but intradermic tests of delayed hypersensitivity did show greater frequency of anergy in the Alcohol group. For anthropometric parameters, the Alcohol +HCV group displayed the lowest triceps skinfold whereas creatinine – height index evaluation was more preserved in the HCV group. Body mass index, arm muscle area and arm fat area showed that differently from alcohol group, the HCV group was similar to control.

Conclusion: Significant differences were found among the main aetiologies of cirrhosis concerning immunological alterations and nutritional status: better nutrition and worse immunology for HCV and vice-versa for alcohol.

Background

For many years malnutrition has been suggested as an important factor in the onset of alcoholic liver disease [1–3]. The high incidence of cirrhosis and steatosis in desti- tute populations, the common malnutritional state of the alcoholics, the association between the severity of the alcoholic liver disease and the degree of malnutrition and the experimental production of steatosis and liver

cirrhosis upon methyonine- and choline-depleted diets strongly support such correlation [3,4].

On the one hand, malnutrition is worse in alcoholics from lower social classes, as a direct consequence of the poor nutritional condition [5]. On the other hand, it is well established that alcoholism, *per se*, is the main cause of malnutrition, even with an adequate alimentary supply, due to deficient food intake, anorexia, nausea and vomiting, poor gastrointestinal absorption, inadequate caloric and protein ingestion, alterations in the carbohydrate, protein and fat metabolism and rise in the energetic expenditure resulting from the occurrence of sepsis or ethanol in these patients [6,7]. Malnutrition is yet associated with worse clinical outcomes in cirrhotic patients – leading to complications such as ascites, encephalopathy, hepatorenal syndrome and diabetes [8], besides the association of worse malnutritional status with higher frequency of death [9,10].

In our view [10] and in the view of others [11,12,1,13–15], the cirrhotic patients who are more severely affected by malnutrition are more likely to present complications, such as infectious processes, presumably due to immunological deficiencies in the humoral and cellular responses. Since these alterations lead to an increase in the catabolism, which in turn aggravates malnutrition, a vicious circle is established [15]. As far as we know, nutritional and immunological alterations in cirrhosis due to HCV have not been addressed together. In fact, the great majority of the studies refer to the alcoholic but not to the viral aetiology of cirrhosis [16,17].

To assess both nutritional and immunological alterations in HCV related cirrhotic patients in comparison to alcoholic cirrhosis and evaluate the alcohol and HCV associated group that is very frequent in the clinical practice were the main objectives of this study.

Patients and Methods
Seventy seven cirrhotic patients were prospective and consecutively studied, and were divided in three groups with respect to aetiology: 33 by alcohol (ALC), 20 by alcohol and HCV (ALC+HCV) and 24 by HCV (HCV) and 32 subjects were enrolled as controls.

Patients were from both genders, aged 18 – 65 years. The sex ratio varied among the three groups, being men more predominant in the groups with alcoholism and women more frequent in the HCV and control groups. The mean age was 52 years for the cirrhotic groups and 37.2 years for the control group. To validate the latter group as control, despite the difference in the mean age, a statistical analysis using age as co-variable was employed in the evaluation of

all parameters; glycaemia was the only one to present a linear correlation with increasing age.

Cirrhotic patients were classified as Child-Pugh A and B, without ascite and/or edema upon physical and ultrasound examination. The control group included non-cirrhotic patients with dyspeptic complaints and for whom general evaluation, ultrasound and endoscopy, showed no alterations or enantematic gastritis only.

Concerning the epidemiological background, there was a higher prevalence of prior surgery (75%) and transfusion (58.33%) within the HCV groups, and no difference between the ALC and ALC+HCV regarding daily alcohol intake, years of consumption or abstinence period.

Patients who had a minimum pure ethanol intake of 80 g/day for at least 8 years were considered chronic alcohol drinkers. Note that patients from the HCV and control groups claimed either no alcohol intake or less than 10 g/day. All patients were inquired for risk factors of viral infections. Patients with associated chronic diseases were excluded (chronic renal failure, congestive heart failure, chronic pacreatitis), as so were those with any other disease leading to malnutrition or poor nutritional absorption, such as neoplasias and hepatocarcinoma. Patients with history of anti-viral treatment for HCV or HIV-positive were also excluded.

Serological detection of the hepatitis B and C viruses and HIV were performed with ELISA tests: second generation anti-HCV KIT from Abott and Ortho Diagnostics; anti-HBc, HBs Ag and anti-HIV from Abott.

All patients were submitted to hepatic function tests by monitoring the following markers: aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, prothrombin time and activity (PTA), hemogram, platelets, seric iron, transferrin, alpha-fetoprotein and glycaemia. Liver biopsy was performed in all patients who had no blood dyscrasia (75,32%) and no other contraindications. Clinical and biochemical criteria according to Garcia-Tsao et al (1985) were considered for diagnosis of hepatic cirrhosis in cases without liver biopsy.

Nutritional evaluation was based on the biochemical parameters – serum albumin and transferrin and anthropometric parameters – actual weight as a percentage of ideal body weight (%IBW), body mass index (BMI), percentage of the standard value for triceps skinfold (% TSF), arm muscle area (AMA), arm fat area (AFA), percentage of TSF and AMC depletion, besides creatinine-height index (CHI).

A single observer, applying the Frisancho rules [19,20] performed the objective-nutritional evaluation. To estimate depletion percentile, the rules of Blackburn et al [21] were employed. The BMI was assessed as described by Heetderks [22]. The classification of the degree of malnutrition was based on the following studies: Blackburn and Thornton [23], Frisancho [19] and Heetderks [22].

The humoral-immunological alterations were evaluated by assessing serum immunoglobulins (A, M, G and E) and the complement – components' 3 and 4 by the Nephelometria methodology. Cellular immunity was evaluated by counting total lymphocytes in the automated counter Cell-Dyn 3000. The peripheral T-lymphocytes and sub-populations – CD4+ and CD8+ were analysed by Flow Cytometry with triple-immunofluorescence staining; the monoclonal antibodies were obtained from Becton-Dickison kit and an Ortho-Diagnostic Cytometer. All patients were subjected to intradermal test for delayed hypersensitivity with the following antigens: purified protein derivative (PPD), candidin, trichophytin and dinitrochlorobenzene (DNCB); the injections were given in the forearm. After 48 hours, reactivity was analysed by measuring the formation of touchable nodules, considered positive if diameter was at least 5 mm. The DNCB test was performed as described by Catalona *et al*, (1972).

The results of the intradermic tests were taken as immuno-competent, those with 2 or more positive tests, hypoergic, those with one positive test only, and anergic, those with no positive reactivity.

The protocol was previously approved by the Ethical Committee of the Hospital and all patients were aware of the research objectives and signed their informed consent.

Statistical Analysis
The Pearson's chi-square and Fisher's exact tests were applied to the four groups in the analysis of the qualitative variables. The remaining variables were initially compared by the univariate analysis ; and the variables belonging to the same class were further studied by the Pearson's-correlation coefficient (r). Whenever correlation between two or more variables was significant, the multivariate analysis was applied further.

Groups were compared regarding their epidemiological data and variables from the groups, that showed significant differences were considered as co-variables. A logarithmic transformation was applied for non normal variable distributions.

Significance was accepted at the 5% probability level (α = 0.05) and the Statistical Analysis System (SAS) was employed in all statistical calculations.

Table 1: Haematological and biochemical results with statistically significant differences in the four studied groups.

PARAMETERS	ALCOHOL			ALC+HCV			HCV			CONTROL			P
	N	M	SD	N	M	SD	N	M	SD	N	M	SD	
Platelets/mm³	33	174×10^3	83×10^3	20	149×10^3	59×10^3	24	127×10^3	70×10^3	32	260×10^3	61×10^3	< 0,001
P.A.(%)	33	68,82	16,21	20	67,60	17,33	24	62,88	13,94	32	87,16	9,17	< 0,001
Iron (mg/100 ml)	33	98,15	30,22	20	99,20	58,97	22	128,45	54,76	31	66,45	32,22	< 0,001
Fasting glycaemia (mg/dl)	33	102,97	25,72	20	126,75	62,57	24	117,67	48,70	32	94,84	10,01	0,003
AST (IU/l)	33	20,42	10,46	20	36,70	19,30	24	58,33	30,52	32	12,75	5,44	0,001
ALT (IU/l)	33	17,21	8,03	20	46,95	42,24	24	66,08	37,40	32	13,25	9,31	< 0,001
AST/ALT	33	1,29	0,59	20	0,91	0,34	24	0,96	0,40	32	1,20	0,61	0,026
Total bilirrubin (mg/dl)	33	1,42	1,11	20	1,40	1,16	24	1,75	0,95	32	0,77	0,38	< 0,001
Alpha-fetoprotein (IU/l)	33	3,90	2,50	19	5,56	3,86	23	13,47	10,87	31	3,71	2,54	< 0,001
Serum Albumin (g/dl)	33	4,19	0,53	20	3,85	0,39	24	3,81	0,57	32	4,49	0,35	< 0,001

Where: N = number of cases; M = mean number; SD = standard deviation; HCV = Hepatitis C Virus. Reference values: N° of platelets/mm³: (140 – 450×10^3); P.A.: (70 – 100), Iron (50 – 100); AST and ALT: (18); Total bilirubin: (0.2 – 1.0); alpha-fetoprotein: (10); Albumin: (3.5 – 5.0).

Results

Upon analysis of small groups of cirrhotic patients of different aetiologies we could demonstrate both haematological and biochemical distinctions, separating alcoholic and viral aetiologies.

Table 1 shows the haematological and biochemical data that distinguished the cirrhotic groups. The HCV group presented higher levels of iron, aminotransferases, total bilirrubin and alpha-fetoprotein, in addition to significantly lower platelets' counting and serum albumin levels. Furthermore, the ALC+HCV group presented significantly higher glycaemia as compared to cirrhosis of other aetiologies, as is showed in Table 2.

The anthropometric parameters as well as their uni- and multivariate analysis for the four groups are presented in Table 3 and 4. The HCV group has shown significantly higher CHI values when compared to the ALC+HCV group, and a marginal difference in comparison to the ALC group, indicating a better nutritional status for the HCV group. Moreover, the associated group, ALC+HCV, has shown the lowest TSF mean values. It should be noted that the HCV was the only group nutritionally distinct from the other cirrhotic groups.

Table 5 shows the uni- and multivariate analysis of nutritional evaluation of the cirrhotic groups using: a) a nutritional evaluation indexes that correlates weight and height (BMI), b) a parameter to evaluate the muscular compartment (AMA), and c) a parameter that evaluates the lipidic compartment (AFA). It also shows that the HCV group had values quite different from other aetiologies of cirrhosis with mean values closer to those of the control group.

Table 2: Multiple comparison with significant results among different cirrhotic aetiologies

| | PARAMETERS | | | | | | | | |
GROUPS	N° Plat.	Iron	AST	ALT	AST/ALT	α-feto	Albumin	Glic	Total bilirrubin
ALCOHOL × ALCOHOL + HCV × HCV	0.04	0.03	0.0001	0.0001	0.01	0.0001	0.004		
ALCOHOL × ALCOHOL + HCV			0.0001	0.0001	0.01		0.01	0.02	
ALCOHOL × HCV	0.01	0.01	0.0001	0.0001	0.02	0.0001	0.003		0.03
ALCOHOL + HCV × HCV		0.03	0.001	0.01		0.0004			

Where: N° plat. = N° of platelets; α-feto = alpha-fetoprotein; Glic. = fasting glycaemia

Table 3: Results of anthropometric parameters of the nutritional evaluation in the four groups by univariate analysis.

PARAMETERS	ALCOHOL			ALCOHOL+HCV			HCV			CONTROL			P
	N	M	DP	N	M	DP	N	M	DP	N	M	DP	
% IBM	33	112.19	19.78	20	107.08	18.00	24	118.45	14.92	32	107.08	19.93	0.103
BMI (Kg/m²)	33	25.75	4.30	20	24.70	3.98	24	26.49	3.28	32	24.00	4.33	0.112
TSF	33	9.36	5.42	20	9.25	3.27	24	11.79	3.12	32	12.88	5.92	0.004
% TSF deplet.	33	71.15	39.46	20	71.67	24.87	24	76.13	18.78	32	83.35	32.75	0.406
AMC	33	26.36	2.76	20	26.22	2.64	24	26.07	3.17	32	24.93	2.93	0.195
% AMC deplet.	33	-4.44	12.51	20	-3.28	12.91	24	-10.19	14.20	32	-4.33	10.79	0.217
AMA (mm²)	33	6.458.89	3.105.85	20	6.235.15	2.933.54	24	7.254.22	3.372.00	32	5.794.70	2.536.05	0.343
AFA (mm²)	33	-869.33	2.013.75	20	-712.69	1.857.64	24	-1.768.83	2.155.96	32	-784.14	1.442.71	0.170
CHI	31	61.59	21.00	20	55.50	25.57	23	80.44	45.90	29	103.27	44.97	0.001

Where: % IBW = percentage of ideal body weight; BMI = body mass index; AMC = arm muscle circunference; % AMC deplet. = percentage of AMC depletion; TSF = triceps skinfold; % TSF deplet. = percentage of TSF depletion; AMA = arm muscle area; AFA = arm fat area; CHI = creatinine height index. **Note:** The ALC+HCV group presented the lowest TSF mean values, while the HCV group showed the highest values for CHI. In the multivariate analysis, only HCV group was nutritionally distinct from other cirrhotic group.

Table 4: Results of the uni and multivariate analysis of the anthropometric parameters on the nutritional evaluation of cirrhotic groups.

Univariate Analysis										
Hypothesis	IBW	BMI	AMC	AMC deplet.	TSF	TSF deplet.	AMA	AFA	CHI	Multivariate Analysis
Four equal groups	0.103	0.112	0.195	0.217	0.004	0.406	0.343	0.170	0.001	0.001
Three equal cirrhotic groups					0.019				0.059	0.001
ALC = ALC + VHC					0.027				0.557	0.771
ALC = HCV					0.648				0.061	0.001
ALC + HCV = HCV					0.006				0.026	0.002

Where: % IBW = percentage of ideal body weight; BMI = body mass index; AMC = arm muscle circunference; % AMC deplet. = percentage of AMC depletion; TSF = triceps skinfold; % TSF deplet. = percentage of TSF depletion; AMA = arm muscle area; AFA = arm fat area; CHI = creatinine height index.

Table 5: Uni and multivariate analysis of the anthropometric parameters of general evaluation (BMI) and muscular (AMA) and lipidic (AFA) compartments.

UNIVARIATE ANALYSIS				
HYPOTHESIS	BMI	AMA	AFA	MULTIVARIATE ANALYSIS
FOUR EQUAL GROUPS	0,112	0,343	0,170	0,001
THREE EQUAL CIRRHOTIC GROUPS				0,001
ALC = ALC + HCV				0,812
ALC = HCV				0,001
ALC + HCV = HCV				0,001
ALC = CONTROL				0,001
ALC + HCV = CONTROL				0,003
HCV = CONTROL				0,089

Note: The HCV group, with no alcohol, was significantly different from other aetiologies being closer to the control group.

Table 6: Humoral-immunological parameters of the four studied groups.

PARÂMETERS	ALCOHOL			ALCOHOL + HCV			HCV			CONTROL			P
	N	M	SD	N	M	SD	N	M	SD	N	M	SD	
C_3 (mg%)	33	106.48	42.72	20	92.62	33.72	24	79.33	26.42	32	123.19	33.16	< 0.001
C_4 (mg%)	33	20.79	8.12	20	19.70	8.57	24	14.21	4.66	32	26.84	7.75	< 0.001
Gammaglobulin (g%)	33	1.54	0.39	20	2.04	0.41	24	2.28	0.76	32	1.35	0.28	< 0.001
IgA (mg/dl)	30	577.27	286.26	20	519.15	203.92	23	505.39	314.59	32	268.94	121.05	< 0.001
IgM (mg/dl)	30	238.33	146.46	20	248.20	96.10	23	325.70	222.32	32	170.03	69.25	0.001
IgG (mg/dl)	30	1.775.87	464.42	20	2.352.10	496.62	23	2.643.96	907.37	32	1.594.88	379.22	< 0.001
IgE (IU/dl)	29	5.80	0.98	18	4.83	1.18	18	3.78	0.81	32	4.65	1.38	< 0.001

Where: C_3 and C_4 are serum complement components 3 and 4, respectively; IgA, IgM, IgG and IgE are immunoglobulins A, M, G and E, respectively. Range for reference values: C_3 (74–185); C_4 (16 – 44); Gammaglobulin (0.8 – 1.6); IgA (153 – 359); IgM (73 – 171); IgG (952 – 1538); IgE (up to 100).

Table 7: Multiple comparisons with significant results among various aetiologies of cirrhosis.

GROUPS	PARAMETERS					
	C_3	C_4	Gamma globulin	IgM	IgG	IgE
ALC × ALC+ HCV × HCV	0.01	0.004	0.0001	0.06	0.0001	0.0001
ALC × ALC+ HCV			0.0001		0.0008	0.005
ALC × HCV	0.004	0.001	0.0001	0.02	0.0001	0.0001
ALC + HCV × HCV		0.01		0.07		0.007

Where: ALC = alcohol; HCV = Hepatitis C Virus; C_3 and C_4 are serum complement components 3 and 4, respectively; IgM, IgG and IgE are immunoglobulins M, G and E, respectively.

Table 8: Immunological results of cellular parameters in the four studied groups.

PARÂMETERS	ALCOHOL			ALCOHOL + HCV			HCV			CONTROL			P
	N	M	SD	N	M	SD	N	M	SD	N	M	SD	
Leukocytes/mm^3	33	5.890.91	1.740.04	20	5.980.00	2.694.80	24	4.966.67	1.705.40	32	6.987.50	1.885.04	0.003
Lymphocytes/mm^3	33	1.527.36	593.11	20	1.929.10	1.167.07	24	1.535.71	725.34	32	2.027.75	619.94	0.021
CD$_4^+$/ul	32	664.44	291.94	19	777.16	450.14	20	663.85	310.07	26	762.54	232.89	0.460
CD$_8^+$/ul	32	297.03	200.15	19	387.00	335.10	20	337.60	315.41	26	439.73	149.79	0.169
CD$_4^+$/CD$_8^+$	32	2.70	1.27	19	2.56	1.08	20	2.50	1.08	26	1.94	0.84	0.064

Where: N° = number of cases; M = mean number; SD = standard deviation; ALC = alcohol; HCV = Hepatitis C Virus. Reference values: N° of leukocytes/mm^3 ($5 - 10 \times 10^3$); N° of lymphocytes/mm^3 ($1 - 3 \times 10^3$); CD4$^+$ ($355 - 1298$); CD8$^+$ ($144 - 796$).

Table 9: Responses to intradermic tests of delayed hypersensitivity from the four studied groups.

GROUPS	N	TWO OR MORE POSITIVE TESTS	ONE POSITIVE TEST	NO POSITIVE TEST
ALCOHOL (%)	32	22 (68.75)	2 (6.25)	8 (25.00)
ALCOHOL + HCV (%)	20	17 (85.00)	2 (10.00)	1 (5.00)
HCV (%)	24	14 (58.33)	8 (33.34)	2 (8.33)
CONTROL (%)	32	26 (85.25)	6 (18.75)	0 (0.00)

$p < 0.001$

The analysis of humoral immunological parameters (Table 6) reveals that the HCV group presents the lowest mean values for the C3 and C4 among the cirrhotic groups. It is also shown that for those groups infected with HCV the gamma globulin mean values were significantly higher than those for the Alcohol group. Regarding the immunoglobulins IgM and IgG, all of the HCV-related groups have shown mean values significantly higher than have the alcohol group. On the other hand, the Alcohol group had the higher IgE values as compared to the other aetiologies of cirrhosis, as is presented in Table 7.

Table 8 shows that while the number of leukocytes and lymphocytes in peripheral blood of cirrhotic patients were significantly lower as compared with the control group, counting of the leukocytes/ lymphocytes did not allow discrimination between the various aetiologies of cirrhosis. Counting of the lymphocytes sub-population CD4+

and CD8+ did not distinguish a single studied group, not even the control, either in the uni. or the multivariate analysis. Only the CD4+/CD8+ ratio was higher for cirrhotic – than control groups.

The analysis of the cirrhotic groups regarding their response to intradermic tests of delayed hypersensitivity is shown in Table 9. It can be seen that the alcohol group displayed a significantly higher percentage of anergic patients than other studied groups (p < 0.001); this pattern was preserved even if the control group was excluded from the analysis (p = 0.02). The HCV group, in turn, showed the highest percentage of immunodeficient patients.

Discussion

The novelty and major contribution of the present study is to open the possibility of discriminating the main different aetiologies of cirrhosis according to nutritional and immunological parameters. The patients with alcoholic cirrhosis in the initial compensated form of the disease show already some degree of malnutrition, as revealed by the different nutritional parameters described by various authors [2,9,25–27].

On the other hand, the HCV-cirrhotic group has shown better values for nutritional parameters, despite the concomitant and conspicuous biochemical alterations. Therefore, it was not possible to correlate higher levels of aminotransferases and bilirrubin, as well as the lower levels of albumin and platelets, with the observed nutritional alterations.

The CHI is probable the most widely used index for evaluating body muscle mass [7], as it correlates with oxygen consumption and lean muscle mass [23]. The CHI was more elevated in the HCV group, showing no association with gender and other parameters used for the evaluation of the muscular compartment, similar to the study of Caregaro and collaborators (1996).

Mean values of TSF being significantly lower in the ALC+HCV group can be interpreted as very specific in the estimation of the fat compartment, since this index is not influenced by liquid retention [28]. Contrariwise, in this same group (ALC+HCV), the other index that allegedly evaluates the fat compartment, named arm fat area (AFA) [19], has not shown a significant alteration in this aetiologic group; as it was also observed by others [29].

As the presence of ascites and/or edema may interfere in the nutritional evaluation of cirrhosis, a special care was taken in terms of excluding such patients from the study, similarly to what was made by other authors [25,28,30].

It is well known that nutritional status does exert an influence in all aspects of immunity, including humoral response, phagocytosis, complement components and, specially, cellular mediated response. As a rule, the effect of caloric and protein depletion in the organism is to suppress or decrease the immune response, although in certain cases malnutrition stimulate immune response. This can be observed, particularly, in the humoral response of patients with alcoholic liver disease or in the response to infections of undernourished patients [31]. The exaggerated humoral response does not mean a higher degree of protection; on the contrary, it is rather a reflex of the dysfunction of the regulatory and stimulatory factors of the immune system [14].

Similarly to the nutritional evaluation, the immunological parameters in the course of cirrhosis were preferentially evaluated in alcoholic aetiology [2,12,32–34]. In our comparative study, the behavior of the lymphocytes mediating the nutritional and immunological alterations was similar in alcoholic and HCV groups. A possible explanation for this similarity in the lymphocyte counting may be due to the fact that the better nutritional state is compensated with the higher immunological alteration and vice-versa in the HCV and alcoholic aetiologies, respectively.

Among the evaluated immunological parameters, there has been observed the decrease in the complement components, fraction 4 in particular, associated with the opsonic and bactericide activity of the cirrhotic sera [35–37]. One could then speculate that lower levels of C4 would lead to higher frequency of infectious processes.

One of the most common immunological alterations in cirrhotic patients is the occurrence of hypergammaglobulinemia [38], with variable increase in IgG, IgA and IgM in different hepatic disorders [39]. In patients with alcoholic cirrhosis there has been shown elevated levels of IgA and IgG [40–42], and normal IgM [42]. Contrariwise, we have observed equal levels of IgG in the alcoholic-cirrhotic and control groups, and elevated levels of IgG and IgM in the HCV-cirrhotic patients. Since many of the former studies with alcoholic cirrhosis were carried out before the HCV detection, it is difficult to rule out an eventual aetiologic association. The elevated levels of IgM and IgG in HCV cirrhosis, described by Sarin and collaborators (1997), could be due to the dysfunction of the T cells, currently taken as responsible for the virus persistence, as well as the severity of the hepatic disease [43]. The elevated levels of IgM found in the HCV group in our study corroborate with Sarin's et al findings [17].

The increase in the IgE levels, however, cannot be associated with severity of the chronic liver disease. As others

[44], we observed the elevation of this fraction in the alcoholic aetiology, but its significance is not clear. It is interesting to note, however, that the degree of malnutrition and anergy was higher in this very group, factors that may influence the levels of IgE [44,45].

The reduction in the number of lymphocytes and the depression in the response to the intradermic tests peculiar to a delayed hypersensitivity seem to be directly related to the severity of liver damage [1,46–48]. In our study, there was no discrimination among the different aetiologies of cirrhosis according to the lymphocyte subpopulations, CD4+ and CD8+, as seen by others [65]. Even though, the CD4/CD8 ratio has distinguished the cirrhotic group from the control patients, what had been reported for the alcoholic aetiology of cirrhosis [49].

Intradermic test of delayed hypersensitivity with higher percentages of anergy have been described by many authors in alcoholic cirrhosis [1,2,12,33]. Anergy was found more frequently among alcoholic patients, albeit poor intradermic response was equally frequent in HCV and alcohol groups, differently from other findings [16]. It is important to point out that the immunodeficiency of the HCV group was not associated with higher degrees of malnutrition, except for the lower mean values of the albumin which has been related to the anergy of the intradermic tests [12].

The most noticeable laboratorial alterations of the HCV group could be related to a deficient cellular immune response. This non-nutritional factor may probably be connected with the severity of the hepatocellular lesion or to immunological alterations triggered by the HCV. These hypotheses demand further investigations to be confirmed.

List of Abbreviations
% IBW, percentage of ideal body weight; BM index, body mass índex; TSF, triceps skinfold; % TSF, percentage of the standard value for triceps skinfold; AMC, arm muscle cirlcunference; AMA, arm muscle area; AFA, arm fat area; CHI, creatinine-height index; CD4+and CD8+, lymphocytes subpopulations, HCV, hepatite C virus; ALC, alcoholic cirrhosis; ALC+HCV, cirrhosis due to alcohol plus HCV virus; IgA, IgM, IgG and IgE, immunoglobulins A, M, G and E.

Competing Interests
None declared.

Contribution of Authors
WRC: conceived of the study, participated in its design, nutritional and immunological evaluation, filling in the protocols and writing the manuscript, ES: design of the study, coordination, writing and editing the manuscript, FJC: design of the study and data analysis AAL: design of the study.
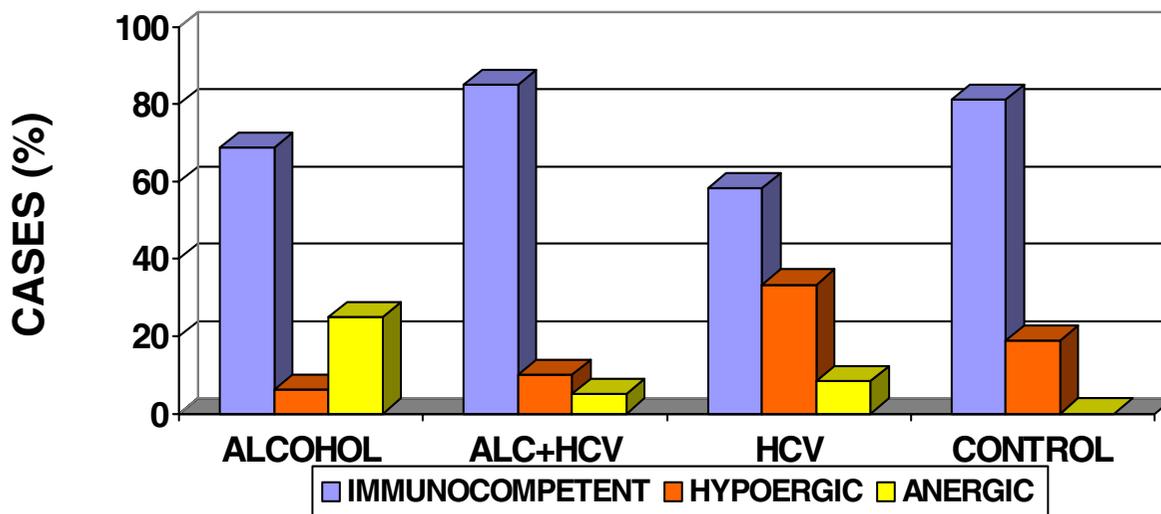
Figure 1
Percentage of immunocompetent, hypoergic and anergic patients according to the intradermic tests of delayed hypersensitivity in the four studied groups.

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