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Hepatitis C Virus-positive patients with persistently normal serum transaminase: a long-term follow-up

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Abstract. AIM: A number of patients with serum hepatitis C virus (HCV) infection have persistently normal alanine aminotransferase (PNALT) levels and some of them may be suffering from liver cirrhosis. This study prospectively evaluated the progression of liver disease in a group of anti-HCV-positive patients with persistently normal ALT levels. PATIENTS AND METHODS: The patients selected for this study had presented in the years 1995/96 with anti-HCV positivity and PNALT according to the AISF criteria and undergone liver biopsy. Patients who remained PNALT in the 18 months after biopsy were included in this study and their liver status was evaluated after a median of 126 months using a number of clinical, biochemical and instrumental examinations. They were also divided into two groups according to ALT evolution. Data were analyzed by classical statistical methods (CSM) and, due to the small number of patients, by Implicative Statistical Analysis (ISA). RESULTS: Twenty-eight patients met the inclusion criteria; thirteen of them remained PNALT, but two of them developed liver cirrhosis (LC). Fifteen patients had flares of ALT (hyperALT) and three of these developed LC. Ten patients with elevated ALT underwent current antiviral treatment and 6 of them were SVR, while 4 were NR. At baseline, the 5 patients who progressed to LC, had age, BMI and arterial hypertension values significantly higher than patients without LC, as well as a more severe degree of grading and staging at histology. In the LC patients there was also a significantly higher number of anti-HBc positive subjects. Comparing patients with flares of transaminases with and without LC, we found a significant difference at baseline for age, BMI, HDL-C, grading and staging. Using ISA on the presence of co-factors of disease, i.e. elevated BMI/obesity, coexistent diabetes mellitus, arterial hypertension and alcohol intake, only anti-HBc positivity showed a statistically significant association with progression of liver disease. The ISA allows us to work on inferential statistics with small samples providing an acceptable significativiy. Another important observation was the implication of diabetes as a cause for reducing antiviral therapy dosage. CONCLUSIONS: in HCV-RNA positive patients associated with

persistently normal ALT levels, the degree of disease activity increases over the years in only half of patients and progression to cirrhosis is slow. Analysis of the results using ISA confirmed the data obtained with traditional methods. Therefore our data, although limited, seem to confirm that liver disease will progress in subjects with a higher degree of liver fibrosis at baseline. The presence of co-factors, i.e. BMI, diabetes mellitus, arterial hypertension, alcohol consumption and HBcAb positivity, was significantly associated with progression, even when the data were analyzed by ISA. Another important point emerging from ISA is the implication of diabetes as a cause for reducing antiviral therapy dosage. The introduction of additional variables and their analysis with the ISA confirm these findings.

Keywords: persistently normal alanine aminotransferase, hepatitis C virus, Implicative Statistical Analysis, supplemetary variables.

Riassunto. Introduzione: Pochi pazienti affetti dal virus dell'epatite C (HCV) presentano livelli di transaminasi persistentemente normali (PNALT) e alcuni di loro evolvono in cirrosi epatica. Abbiamo valutato prospetticamente la progressione della malattia epatica in un gruppo di pazienti con PNALT. PAZIENTI E METODI: Sono stati valutati pazienti che presentavano anti–HCV positività e PNALT secondo i criteri dell' AISF nel 1995/96 e sottoposti a biopsia epatica. Venivano inclusi i pazienti che mantenevano le PNALT nei 18 mesi post-biopsia, valutando la loro funzionalità epatica dopo una mediana di 126 mesi. Essi venivano suddivisi in due gruppi in base all' andamento delle ALT. La valutazione includeva un certo numero di esami clinici, biochimici e strumentali per stabilire l'eventuale progressione della malattia epatica. I dati venivano analizzati secondo metodi statistici classici (CSM) e, dato il piccolo numero di pazienti, dalla analisi statistica implicativa (ISA). RISULTATI: 28 pazienti soddisfacevano i criteri di inclusione; tredici di essi mantenevano PNALT, ma due di loro sviluppavano la cirrosi epatica. Quindici pazienti avevano dei flares delle ALT (hyperALT) e tre di loro sviluppavano LC. Dieci pazienti con ALT elevate costantemente erano sottoposti ad un trattamento antivirale. 6 avevano una SVR, mentre 4 erano NR. Al basale, i 5 pazienti con LC, avevano l'età, BMI e valori della pressione arteriosa significativamente più alti rispetto ai pazienti senza LC, così come una classificazione istologica (grading e staging) più severa. Nei pazienti con LC c'era anche un significativo numero più elevato di soggetti antiHBc positivi. Confrontando i pazienti hyperALT con e senza LC, vi era una differenza significativa al basale per età, BMI, colesterolo HDL, grading e staging. Utilizzando l' ISA la presenza dei cofattori di malattia, cioe il soprappeso/obesità, la coesistenza di diabete mellito, di ipertensione arteriosa, di consumo alcolico, di positività dei marcatori di HBV, anche la sola presenza dell'anti-HBc sono risultati statisticamente significativi. L'ISA ci permette di lavorare su una statistica inferenziale con piccoli campioni garantendo una significatività accettabile. Altro dato che si evince è la implicazione del diabete nella riduzione dei dosaggi della terapia antivirale. CONCLUSIONI: Nei pazienti PNALT, il grado di attività della malattia aumenta nel corso degli anni solo nella metà dei pazienti e la progressione a cirrosi è lenta. L'Analisi dei risultati ottenuta dall' ISA conferma i dati ottenuti con CSM. Pertanto, dai nostri dati, sebbene limitati, sembra confermare che i soggetti con più

elevato grado di fibrosi epatica al basale sono quelli che evolveranno la loro malattia. La presenza di co-fattori: BMI, diabete mellito, ipertensione arteriosa, consumo di alcool, anti HBc positività erano significativamente associata con la progressione, anche quando i dati sono stati analizzati con ISA. Un punto importante che emerge da ISA è la implicazione del diabete nella riduzione dei dosaggi della terapia antivirale. L'introduzione di variabili supplementari e le relative analisi con l'ISA confermano questi risultati.

Parole Chiave: transaminasi persistententemente normali, epatite c, analisi statistica implicativa,variabili supplementari.

INTRODUCTION: Un certain nombre de patients atteints d'hépatite C (VHC) ont des taux d'aminotransaminase normaux de façon persistante (PNALT) et quelques-uns d'entre eux peuvent évoluer vers la cirrhose du foie. Nous avons évalué de façon prospective l'évolution de la maladie du foie dans un groupe de patients anti-HCV positive. PATIENTS ET METHODES. Nous avons évalué les patients qui présentaient en 1995-1996 un anti-HCV positif, un PNALT selon les critères de l'AISF et une biopsie du foie. Les patients qui ont conservé le PNALT dans les 18 mois après une biopsie du foie appartiennent à cette étude. L'évaluation de leur fonction hépatique, dans une moyenne de 126 mois, est faite après usage d'un nombre d'examens cliniques, biochimiques et d'interventions instrumentales. Ils ont été divisés en deux groupes en fonction de leur ALT. Les données ont été analysées selon des méthodes statistiques classiques (CSM) et, étant donné le petit nombre de patients, avec l'analyse statistique implicative (ASI). RÉSULTATS: 28 patients répondaient aux critères d'inclusion, treize d'entre eux gardaient un taux PNALT normal, mais deux d'entre eux ont développé une cirrhose du foie. Quinze patients ont eu des poussées de ALT (hyperALT) et trois d'entre eux ont développé LC. Dix patients, avec une ALT élevée, ont été traités en continu par un traitement antiviral. 6 avaient une SVR, tandis que 4 ont été NR. Au départ, 5 patients qui ont accentué LC, étaient âgés, avec un BMI et une tension artérielle significativement plus élevés que les patients sans LC, ainsi qu'un degré histologique plus grave. Chez les patients LC on note un nombre significativement plus élevé de sujets anti-HBc positive. En comparant les patients en poussée de transaminases avec et sans LC, il y avait une différence significative selon l'âge, le BMI, le cholestérol HDL, la classification. L'utilisation de l'ASI en présence des cofacteurs de la maladie, par exemple le surpoids ou l'obésité, la

coexistence d'un diabète sucré, l'hypertension, la consommation d'alcool, des marqueurs du VHB positifs, la seule présence d'anticorps anti-HBc a été statistiquement éclairante relativement au progrès de l'affection du foie. L'ASI permet de travailler sur l'inférence statistique sur de petits échantillons tout en fournissant une signification acceptable. Une autre observation montre l'implication du diabète comme cause de la réduction des doses de la thérapie antivirale. CONCLUSIONS: Chez les patients PNALT avec une association à des niveaux ALT élevés, le degré d'activité de la maladie augmente au fil des ans seulement pour la moitié des patients et l'évolution vers la cirrhose est lente. L'analyse des résultats obtenus à partir de l'ASI confirme ceux obtenus avec les méthodes traditionnelles. Par conséquent, nos données, quoique limitées, semblent confirmer que les personnes au plus haut degré de la fibrose hépatique au départ sont ceux qui développeront la maladie hépatique. La présence de cofacteurs, par exemple le BMI, le diabète sucré, l'hypertension, la consommation d'alcool, la positivité de l'HbcAb, était significativement associée à la progression, même lorsque les données sont analysées par l'ASI. Un point important qui ressort de l'ASI est l'implication du diabète dans la réduction des doses de la thérapie antivirale. L'introduction de variables supplémentaires et leur analyse avec l'ASI confirment ces résultats.

Mots-clés: transaminases persistantes normales, hépatite C, analyse statistique implicative, variables supplémentaires.

1 1. INTRODUCTION

In patients with chronic hepatitis C virus (HCV) infection, 30-40 % show persistently normal alanine aminotransferase (PNALT) levels [1,2]. Although formerly referred to as 'healthy' or 'asymptomatic' HCV carriers, the course of HCV infection is not so simple as would appear, given that the evolution of liver disease in a part of these subjects is less benign than previously believed. It has now become clear that the majority of these patients have some degree of histological liver damage, which may be significant in up to 20% of patients and might even progress toward the more severe degrees [4-19].

A critical point in these subjects is the definition of the "persistent" normality of ALT serum levels. In fact, during HCV infection it is not rare to observe great fluc-

tuations in ALT, which may remain normal for months or years to rise quickly in a few cases, in association with a more severe histological picture [2]. The Italian Association for the Study of the Liver (AISF) proposed some years ago that the time length to define a PNALT carrier should be 18 months, including a total of 9 assays of serum ALT with a 2-month time lapse between each assay, which means that a single increase in ALT values above normal in one out of nine assays excludes patients from this category [9].

In the past, HCV carriers with PNALT had been excluded from antiviral treatment [2], then subsequently some studies showed that the rate of virological response in these patients is similar to that observed in patients with elevated transaminase [20-23], so that currently antiviral treatment has also been indicated for PNALT carriers, although with some limitations [24, 25]. However, to date it is not clear which of them are at risk for disease progression and, therefore, whether it is worth treating them with antiviral therapy [24] .

The aim of this study was to report the clinical history of a group of subjects, labeled as carriers of PNALT according to the literature [9], who underwent liver biopsy in the years 1995-1996 and were followed up for an average of 15 years at our center. They were divided into two groups according to transaminase levels during the 18 months following liver biopsy: those who maintained normal ALT and those with elevated ALT values. The aims were to evaluate: a) how many patients were PNALT for a long period of time but eventually developed liver disease; b) the stage of liver disease in patients who had elevated serum ALT and c) what factors could have influenced the progression of liver disease.

Moreover, as a secondary point, we discussed the cost-effectiveness of treatment.

2 PATIENTS and METHODS

2.1 .PATIENTS

The total study population included subjects extrapolated from our previous studies on patients with PNALT [26, 27] who were still being followed-up in our outpatients clinic for liver diseases. They all had serum transaminase levels persistently within normal limits, defined according to the accepted criteria at that time [28, 29]**,** and had undergone liver biopsy in the years 1995/96. Seventy patients met these criteria, 17 were excluded because they were HCV-RNA negative at baseline. The remaining were carefully followed up by monitoring transaminase levels every two months for 18 months after liver biopsy. At the end of this period, 18 patients

had had flares of ALT, 7 had dropped-out and 28 maintained normal ALT levels, so only these 28 patients were finally included in this study. Moreover, they were divided into two groups according to serum transaminase levels in the 18 months after liver biopsy, those who maintained values and those with elevated ALT. The current assessment of patients also included a median follow-up of 126 months (range 122-148 months).

2.2 METHODS

On entry to the study, the patients underwent a general examination including evaluation of body weight, height, Body Mass Index (BMI), blood pressure and heart rate. The main parameters of liver function as well as the lipidemic pattern were evaluated using commercial kits and markers of hepatitis B virus and qualitative HCV-RNA tests were also repeated. Subjects with alcohol consumption were excluded at enrolment, as previously described [8]. Patients also underwent instrumental diagnosis, i.e. ultrasound of the upper abdomen and color-Doppler of the liver and spleen to help determine current liver status. Furthermore, two non-invasive current markers of liver fibrosis were used i.e. transient elastography and APRI score, the latter being compared to the same score calculated at liver biopsy.

2.2.1 Ultrasound and color Doppler

Ultrasound of the liver and color Doppler of the portal vein and spleen were performed by two operators in the morning after fasting for at least 10 h using before 2001 a real-time Toshiba SSA 270 A apparatus with a 3.75 MHz convex and 5 MHz linear probe, and after 2001 a real-time Philips 5000 HDI apparatus with 2-5 MHz, convex, multi-frequency and 12-5 MHz, linear, multi-frequency probes. The linear probe was used to assess the liver surface. The ability of the two ultrasound observers (GM, MS) was homogeneous: they had the same professional background, having been trained in this specific field, and both had over a decade of experience. Portal vein flow velocity (time-averaged maximum velocity in cm/sec) and portal vein diameter, measured as the largest antero-posterior caliber at the crossing point with the hepatic artery during suspended respiration as well as splenic artery RI (Resistance index), measured intraparenchymally, near to the hilus [30, 31] were evaluated. We considered as ultrasound signs of cirrhosis an irregular liver surface and signs of portal hypertension (portal vein diameter greater than 1.2 cm, with portal vein flow velocity <24 cm / sec and splenic artery RI <0.64) [30, 31]

2.2.2 Non-invasive markers of liver fibrosis

Transient elastography: After January 2007 liver fibrosis was assessed by a single certified operator (trained by the manufacturer) using TE (FibroScan®; Echo-Sens, Paris, France). TE provides an assessment of liver stiffness expressed in KPa units. In brief, an ultrasound transducer probe is mounted on the axis of a vibrator. Vibrations of mild amplitude and low frequency are transmitted by the transducer, inducing an elastic shear wave that propagates through the underlying tissues. The speed of propagation of this vibration across the liver is directly related to tissue stiffness.

The tip of the probe transducer was placed in the intercostal spaces at the right lobe of the liver. Only patients with 10 valid elastometric measures, interquartile ranges (IQR) > 30% and ≥60% success rate (the number of validated measurements divided by the total number of measurements) were considered to be reliable. A cut-off of 8.3 KPa was used to correctly diagnose subjects with significant fibrosis and a cut-off of 14 KPa to correctly assess liver cirrhosis [32].

AST-to-Platelet-Ratio Index (APRI): Liver fibrosis was also assessed using a well-validated index, the AST to platelet ratio index (APRI), which is calculated as follows: AST / upper limit of normal (ULN) x 100 / platelet count (10^9/L). The prevalence of advanced fibrosis was estimated using an APRI index >1.5 as a reference [33].

Liver biopsy at entry to the study was obtained percutaneously with a Menghini needle and the Histology Activity Index (HAI) was evaluated according to Knodell [34]. Genotyping was performed as previously described [8].

Patients with elevated serum transaminase were treated with current therapies and referred to as SVR or NR according to current European guidelines [28].

Diagnosis of cirrhosis was based on the presence of unequivocal clinical, biochemical and instrumental signs. Diagnosis of arterial hypertension (AH) was made in accordance with WHO/ISH criteria [35]. Diabetes Mellitus or Impaired Fasting Glucose (IFG) was diagnosed according to the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus criteria [36]

2.3 . STATISTICAL ANALYSIS

The data were analyzed using classical statistical methods (CSM) and Implicative Statistical Analysis (ISA) (non parametric); the latter offers the advantage of not being influenced by a number of patients ≤100.

2.3.1 Analysis with classical statistical methods.

When data distribution was Gaussian, values were expressed as mean ± standard deviation and their differences were calculated using Student's t-test, otherwise data were expressed as median and range and analyzed with the Mann-Whitney U test. Fisher's exact test, χ^2 test and Spearman's rank correlations were used when appropriate. Finally, multiple logistic regression analysis was performed to estimate the independence of the association between variables significant at univariate analysis and presence/absence of liver cirrhosis. Variables contributing to significantly fit the logistic equation were then selected by a step-wise procedure. $P< 0.05$ was considered significant. Statistical analysis was performed using the SPSS for Windows version 13.0.

2.3.2 Implicative Statistical Analysis (non parametric) (ISA).

The data were analyzed in a quantitative way, using the software of inferential statistics CHIC 2007 (Classification Hiérarchique Implicative et Cohésitive). This shows three well-defined groups of the experimental variables (with statistical percentages of 95% and 99%). For references on ISA see: Gras R., Guillet F., Spagnolo F**.,** Suzuki E., (2008).

3 RESULTS

Following the initial 18 months after liver biopsy patients were recalled to our Center every 6 months for an examination and evaluation of routine hematochemical parameters, and every 12 months for abdominal ultrasound evaluation. Patients with US signs of liver evolution were followed up with US every 6 months.

3.1 Traditional statistical analysis of data

As indicated above, 28 patients (17M, 11F) who maintained normal serum ALT levels until 18 months after liver biopsy were included in this study. Figure 1 shows the evolution of serum ALT and liver disease during the follow-up. Thirteen patients maintained persistently normal ALT values during the whole period, but two of these developed overt liver cirrhosis. Among the other subjects, only one patient received antiviral treatment, i.e. Peg-IFN + Ribavirin. The choice to treat was made due to the strong determination on the part of the patient to be treated, even though there were no signs of progression of liver disease. At the third month of therapy, HCV-RNA was negative. Four of these patients had genotype 2, two genotype 3 and seven genotype 1b.

In 15/28 patients, however, serum transaminase levels increased during the 18 months post biopsy and three of them developed overt liver cirrhosis. Ten patients underwent current antiviral treatment with six SVR and 4 NR. Among the SVR there were three genotype 1b, two genotype 2 and one genotype 3; the NR patients all had genotype 1b. Two of the three cirrhosis patients were included in the treated patients and one of these two was an SVR. The remaining 5 patients did not receive treatment because of fluctuating serum ALT levels < 1.5 N in three cases (genotype 1,2,3, respectively) and due to a brief temporary increase in ALT in the remaining two (both genotype 1b) likely related to the assumption of other drugs.

Table 1 shows the comparison of some baseline demographic, biochemical and histological characteristics of the 28 patients divided according to ALT evolution. This comparison did not reveal any statistically significant differences.

Table 2 shows some demographic, biochemical and histological characteristics of the patients who developed liver cirrhosis compared to the remaining patients at entry to the study. At baseline, the 5 patients who developed liver cirrhosis had age, BMI and arterial hypertension values significantly higher than patients without liver cirrhosis ($P< 0.005$, $P< 0.05$, $P<0.003$, respectively), while serum HDL-C was significantly lower ($P<0.002$). Grading ($P<0.006$) and staging ($P<0.003$) were more severe and the frequency of HBcAb was significantly higher ($P< 0.02$) in the LC group.

Table 3 shows the same characteristics as Tables 1 and 2 at baseline in patients who had flares of ALT, divided into those with or without LC. Patients who developed overt liver cirrhosis had age and BMI significantly higher ($P<0.03$ and $P<0.05$, respectively), lower levels of HDL-Cholesterol ($P<0.02$) and a more severe liver disease i.e. grading ($P<0.003$) and staging ($P<0.003$) than patients who did not develop frank liver cirrhosis.

At gastro-esophageal endoscopy 3 out of 5 LC patients showed varying degrees of esophageal varices.

Elastography: Twenty out of 28 patients underwent TE; 6 overweight patients, one patient suffering from Parkinson's disease and one pregnant woman were excluded. Two patients showed values above 8.3 KPa (8.6 in one patient with PNALT and LC and 9.6 in a non-cirrhotic patient with high ALT values) and in only one case TE was over 14 KPa (33.8 in a cirrhotic patient with elevated ALT values).

APRI: Table 4 shows the APRI scores at baseline and at the end of follow-up. When patients were evaluated for liver cirrhosis there was a significant increase in score at the end of follow up (ρ=0.3; P<0.04), while when evaluated for fibrosis there was no significant difference.

Multiple logistic regression of variables significant at univariate analysis showed no significant association of these variables with development of liver cirrhosis.

3.2 Data analysis with ISA

3.2.1 Implicative analysis

The implicative analysis of variables to 90% was as follows:. Figure 2 shows a first-step analysis ISA. It is clear that the variables BMI, arterial hypertension (hyper) and IFG / diabetes (DIA) imply the presence of "cirrhosis". This result agrees with other experimental results (analysis of cases or groups of cases).The variable (DIA) in turn implies the variable (red IFN), showing that in diabetic patients the dosage of antiviral drugs is more likely to be reduced.

3.2.2 Cohesive Tree

Figure 3 expresses a second step of the ISA. The data in this graph confirm the results of the previous graph and the resulting implications provide further essential knowledge.

In detail:

1. implication "A" indicates that a person in cirrhotic evolution implies alcohol consumption. This finding was not present in the previous graph.
2. the variable (DIA) implies the variable (red IFN), showing that in diabetic patients a reduction in the dosage of antiviral drugs is more likely as already shown in Fig. 1. The variable (BMI) implies (CIRR), thus a subject with obesity is more likely to develop cirrhosis. The two variables (DIA, CIRR) imply males (Man), thus males are more at risk for cirrhosis than females.
3. the variable (ALT) implies the variable (GGT), and both imply (HBcAb). This means that a person with positive markers of B virus is more likely to have an increase in serum transaminase and gammaGT

3.2.3 Additional Variables and the similarity graph.

Two additional variables to individuals were introduced: (PNALT) and (cirrhosis), which were identified using serum markers of liver function and instrumental tests.

The transposed matrix allowed us to analyze groups of patients with the same characteristics.

The similarity graph (Figure 4) confirmed that patients with hepatitis C and persistently normal transaminase have a low probability of evolving to cirrhosis, the data in the literature reporting 30%.

4 Discussion and Conclusions

In the present study 5 out of 28 HCV-RNA positive patients developed overt liver cirrhosis, confirming that PNALT patients need to be monitored like those presenting elevated ALT levels. This statement is also supported by the fact that in PNALT patients it is difficult to determine those whose liver disease may progress. The prevalence of PNALT subjects with normal liver at biopsy (true "carriers"), as already reported by Montalto et al. [8] and by others in more recent studies [2,6,7,9,10], can be as high as 20% . In most cases there are a varying degrees of liver damage, fibrosis is usually mild or absent [10] and histology is generally less severe than in patients with elevated or fluctuating serum ALT levels [2, 7, 10,11]. Recent studies have shown more severe liver damage (fibrosis $\geq$ F2) in at least 20% of cases and liver cirrhosis in 3-5% of PNALT patients, and the possibility of fibrosis progression in around 20-25% [12-14]. There are also reports of rare cases of hepatocellular carcinoma in patients with normal ALT [15, 16], even though liver structure is histologically normal [17].

Our data, although limited by the small number of patients, appear to confirm that liver disease will progress in subjects presenting a higher degree of liver fibrosis at baseline. Other factors that, in our opinion, may contribute to the progression are the classical co-factors of liver disease, i.e. elevated BMI, presence of diabetes mellitus, arterial hypertension, low levels of HDL-Chol and even only association with HBcAb positivity. Therefore, it is of paramount importance to monitor this particular category of patients and correct any eventual metabolic disturbances (i.e. insulin resistance) to avoid the development of a metabolic syndrome, which would worsen the evolution of liver disease.

Similar data have been reported by Persico M. et al. who studied the natural history of a group of 24 patients with PNALT over 10 years compared with a group of 40 patients with elevated levels of transaminase, using liver biopsy at baseline and after 5 and 10 years. They did not report any significant histological differences in patients with PNALT in the three liver biopsies, confirming that cirrhosis progres-

sion is low or absent in these patients. Presence of liver steatosis was significantly higher in the group with high ALT, confirming that steatosis is a co-factor of disease progression. In this study neither sex nor BMI were significantly different in the two patient groups [18].

The small number of our sample, as well as that of others in the literature, is explained by the fact that to obtain correct information liver biopsies should be performed both at the beginning and at the end of a study, which is not ethically justifiable except in specifically designed controlled studies. One limitation of our study is certainly that a second liver biopsy was not performed after all these years, but as a surrogate we used liver function tests, ultrasound, elastography and clinical examination, avoiding invasive procedures since in the majority of these patients there was no progression of disease. However, it is mandatory to continue monitoring these patients, as we now know from studying the natural history of HCV disease. It may take decades and sometimes longer before HCV infection progresses to cirrhosis and therefore patients with normal liver today may later on show evidence of liver impairment. These considerations, as well as the evidence that combination therapy is just as effective in subjects with normal ALT, once again suggest that there is no substantial difference between the two patient groups, and that the same follow-up and treatment criteria should be applied to subjects with normal ALT as to those with elevated ALT.

PNALT HCV carriers have traditionally been excluded from antiviral treatment, both in trials and in clinical practice [2]. A first therapeutic development occurred with the use of IFN-ribavirin combination therapy, which obtained virologic response rates that did not differ from those seen in patients with elevated transaminase, as evidenced by a study by Mangia et al. and later confirmed by other works [20-23]. More recently, the results of three studies designed to evaluate the efficacy and safety of treatment with pegylated interferon alfa-2a plus ribavirin also appear to confirm these data [1, 25, 41, 42]. This new knowledge was highlighted both in the American Association for the Study of Liver Disease document published in 2004 [11] and in the American Association of Gastroenterology (AGA) document published in January 2006 [19]. The Italian Association for the Study of the Liver in a guideline document on the treatment of hepatitis C has confirmed this new orientation [24]. Following the AIFA (Italian Drugs Agency) evaluation (43), the use of IFN in patients with HCV-related chronic hepatitis has changed, so that treat-

ment with pegylated interferon alfa-2a and ribavirin is currently allowed in HCV carriers regardless of transaminase levels [44].

In view of the not entirely marginal economic costs and the possible side effects of treatment, a careful evaluation of cost-effectiveness and a careful selection of patients referred for treatment is essential.

Possible fundamental criteria for the definition of optimal treatment protocols may be patient age, his/her motivation, possibility of eradication (viral genotype), life expectancy, duration of disease, presence of co-factors of liver disease, treatment compliance, contraindications, considerations about infectiveness (if a subject is promiscuous or has a stable partner), type of job (potential infection of others).

It has been proposed that younger patients (45-50 years maximum), with a "favorable" genotype (genotype 2-3), low viral load, high motivation and without contraindications could start the treatment even without liver biopsy. In these subjects, in fact, the probability of response is so high and the side effects so low that treatment may be hypothesized without preliminary knowledge of a patient's histology. In these cases the "liver biopsy surrogates" could be useful. Liver biopsy could eventually be performed, if necessary, in non-responders to treatment.

On the contrary, in patients in which the cost-effectiveness ratio is not favorable (age over 50, relative contraindications, poor motivation, genotype 1, high viral load, presence of co-factors, risk of side effects, etc.) the opportunity of a treatment should be assessed case by case, according to the severity of liver histology, reserving therapy for those patients with a high grade of fibrosis (>F2) and strictly monitoring subjects with or without moderate fibrosis. Finally, in subjects aged over 60-65 years and with long duration of disease, a clinical follow-up would seem reasonable, thus avoiding both liver biopsy and treatment [24, 39].

As mentioned above, a limitation of our study is the small number of patients examined, but being aware of this, we attempted to support the data obtained with standard statistical methods by using ISA.

ISA allows inferences about a larger population: it is a statistical inference (Gras-Einoshin-Guillet-Spagnolo, 2007). Furthermore, non-parametric statistical methods allow the analysis of small samples. The results obtained by the implicative analysis graph confirm the data obtained by traditional methods and the analysis of cases.

The presence of cofactors of the disease, namely elevated BMI, coexistence of diabetes mellitus, arterial hypertension and HBCAb positivity, were statistically sig-

nificant on ISA. Interestingly, a fact that appears only with this statistical tool, at least from our data, is the implication that the presence of diabetes would affect the dosage of antiviral therapy. Diabetic patients undergoing antiviral therapy seem to be more likely to experience side effects which require a reduction in drug dosage, thus compromising the success of therapy.

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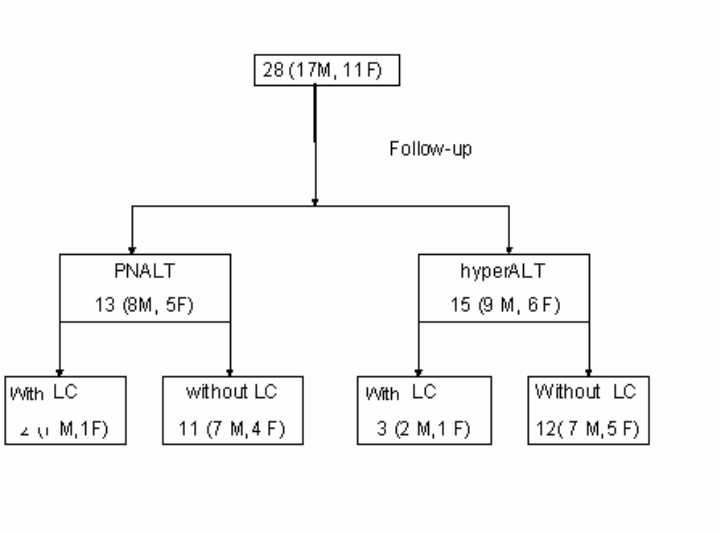

Figure 1: Evolution of the patients studied after 10 years of follow-up

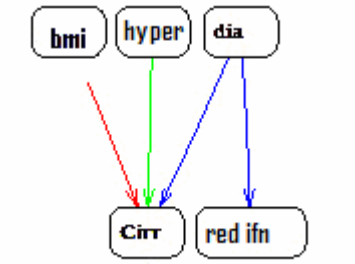

Figure: 2 Implicative analysis

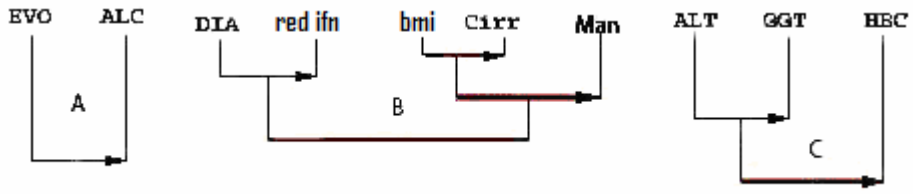

Figure 3 Implicative analysis. The cohesive tree expresses a second step of the ISA. The data in this graph confirm the results of the previous graph and the resulting implications provide further essential knowledge.

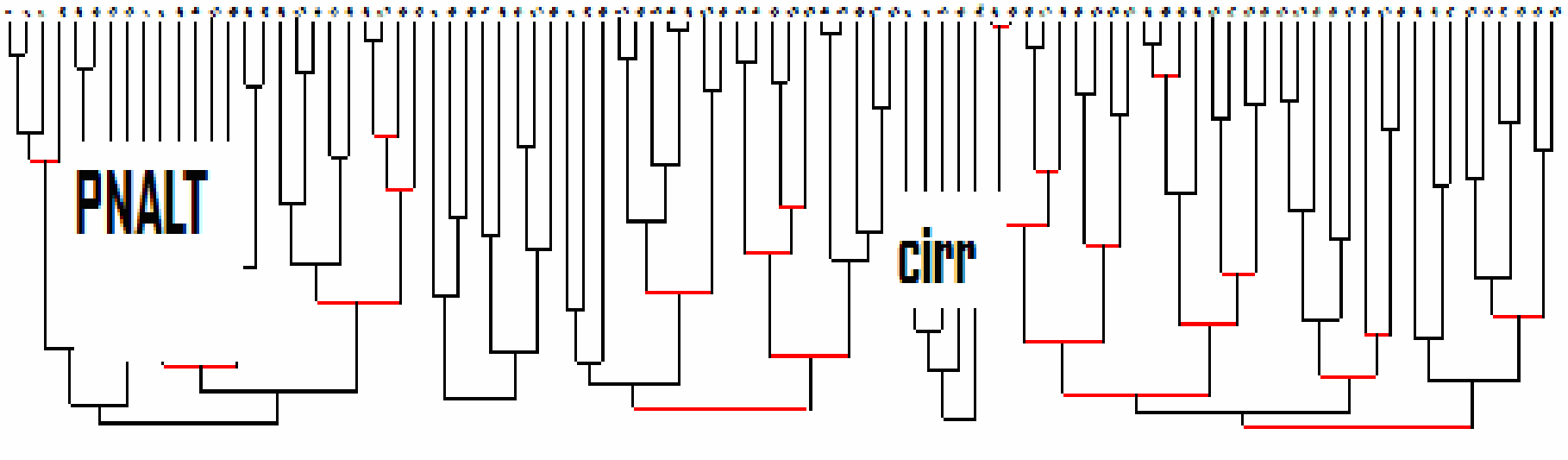

Figure 4 Similarity graph. Additional Variables and the similarity graph

Table 1: Comparison of some baseline demographic, biochemical and histological characteristics of 28 patients divided according to their ALT evolution.

	Hyper ALT N=15	PNALT N=13	P<
Sex (M)	9	8	Ns
Age	41.1 ±14.5	45.1 ± 15.2	Ns
BMI	25.5 ± 3.7	26.2 ± 4	Ns
Alk. Phos. (U/l)	97.5 (46-268)	104 (64-186)	Ns
Gamma-GT (U/l)	26 (8-162)	17 (8-42)	Ns
PT %	94 ± 5	91 ± 10	Ns
Alb	4.4 ± 0.5	4.1 ± 0.5	Ns
Gamma glob. (g/dl)	1.12 ± 0.3	1.5 ± 0.4	Ns
PLT x mmc	375769 ± 65156	251615 ± 91773	Ns
Genotype 1	10	7	Ns
HBcAb	2	0	Ns
Arterial Hypertension	2	0	Ns
IFG or Diabetes	0	0	Ns
Cholesterol (g/dl)	176 ± 40	181.7 ± 25	Ns
Triglyc. (mg/dl)	89.7 ± 40	69.2 ± 11	Ns
HDL-C (g/dl)	52 ± 12	51.7 ± 19	Ns
Staging	1 (0-3)	1 (0-3)	Ns
Grading	5 (0-14)	6 (1-11)	Ns

Table 2: Comparison between characteristics at baseline of patients who developed vs those who did not develop cirrhosis

	Without LC N = 23	With LC N = 5	P<
Sex(M)	15	2	Ns
Age	42.5 ±12.6	52.4 ± 4.1	0.005
BMI	25.9 ± 3	29.2 ± 2.2	0.05
Alk.Phos. (U/l)	111.9 ± 49.3	107.4 ± 48.2	Ns
Gamma-GT (U/l)	23 (8-128)	32 (16-162)	Ns
PT %	91.8 ± 8	100 ± 4.9	Ns
Alb (g/dl)	4.3 ± 0.5	4 ± 0.6	Ns
Gamma glob. (g/dl)	1.3 ± 0.3	1.5 ± 0.3	Ns
PLT x mmc^3	316363±52000	263200 ± 13000	Ns
Genotype 1	13	5	Ns
HBcAb	0	2	0.02
Arterial Hypertension	0	3	0.003
IFG or Diabetes	2	0	Ns
Cholesterol (g/dl)	187.6 ± 35	172 ± 20	Ns
Triglyc. (mg/dl)	90.7 ± 48	69.2 ± 11	Ns
HDL-C (g/dl)	53.2 ± 13	44 ± 18	0.02
Grading	4 (0-11)	11 (5-14)	0.003
Staging	0.5 (0-2)	3 (1-3)	0.006

Table 3: Comparison between characteristics at baseline of patients with liver cirrhosis (LC) vs patients (with hyper ALT) without LC

	Baseline		
	HyperALT Without LC N = 15	LC N = 5	P<
Sex (M)	9	2	Ns
Age	41.1 ±14.5	52.4 ± 4.1	0.03
BMI	25.5 ± 3,7	29.2 ± 2.2	0.05
Alk. Phos. (U/l)	97.5(46-268)	107.4 ± 48.2	Ns
Gamma-GT (U/l)	26(8-162)	32 (16-162)	Ns
PT %	94 ± 5	100 ± 4.9	Ns
Alb	4.4 ± 0.5	4 ± 0.6	Ns
Gamma glob. (g/dl)	1.12 ± 0.3	1.5 ± 0.3	Ns
PLT x mmc^3	429500±77000	263200 ± 13000	Ns
Genotype 1	3	5	Ns
HBcAb	0	2	0.052
Arterial Hypertension	0	2	0.052
IFG or Diabetes	0	0	Ns
Cholesterol (g/dl)	176 ± 40	172 ± 20	Ns
Triglyc. (mg/dl)	89.7 ± 40	69.2 ± 11	Ns
HDL-C (g/dl)	52 ± 12	44 ± 18	0.02
Staging	1 (0-3)	3 (1-3)	0.003
Grading	5 (0-14)	11 (5-14)	0.003

Tabella 4: APRI values at baseline and at the end of follow up

APRI	Interpretation	Baseline	End of follow-up	
<0.5	Absence of significant fibrosis	25	22	
0.5-1.5	Unclassified to significant fibrosis	3	1	ρ=0.15; P = ns
>1.5	Presence of significant fibrosis	0	4	
<1	Absence of cirrhosis	28	24	
1-2	Unclassified to cirrhosis	0	1	
>2	Presence of cirrhosis	0	4	ρ=0.31; P<0.02