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Long-term follow-up of hepatitis C virus-positive patients with persistently normal serum transaminases

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ABSTRACT

Material and methods. This study prospectively evaluated the progression of liver disease in a group of anti-HCV-positive patients with persistently normal ALT levels (PNALT) who were HCV-RNA positive. Patients selected for this study were those who presented with PNALT according to the Italian Association for the Study of the Liver (AISF) criteria in the year 1995/96 and underwent liver biopsy. They were divided into two groups according to their ALT evolution. Forty-five patients were included in this study. **Results.** After a median follow-up time of 180 months twenty-five of them maintained PNALT, but two of these developed liver cirrhosis (LC) in a mean time of 174 and 202 months, respectively. Twenty patients had flares of ALT and three of them developed LC in a mean time of 162-178 months. Twelve of these patients underwent current antiviral treatment; six patients were SVR. At baseline, the 5 patients who progressed to LC had age and BMI significantly higher than patients without LC ($P < 0.005$ and $P < 0.01$, respectively). Grading ($P < 0.006$) and staging ($P < 0.003$) were also more severe at histology, while serum HDL-C levels were statistically lower ($P < 0.002$). 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 ($P < 0.05$; $P < 0.01$ and $P < 0.003$, respectively). **Conclusion.** In HCV-RNA positive patients associated with PNALT the grade of disease activity increased over the years in only half of patients and a higher degree of liver fibrosis at baseline was the major relevant factor for progression.

Key words. Chronic hepatitis C. Persistently normal transaminases. Liver histology. Progression of disease. Interferon treatment.

INTRODUCTION

30-40% of patients with chronic hepatitis C virus (HCV) infection show persistently normal alanine aminotransferase (PNALT) levels.[1,2] Although these were formerly referred to as 'healthy' or 'asymptomatic' HCV carriers, the natural history of HCV infection is not so clear-cut, as the evolution of liver disease in some of these subjects is less benign than previously thought. 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 progress toward the more severe degrees.[4-19]

A critical problem to be considered in these subjects is the definition of the "persistent" normality of serum ALT levels. In fact, during HCV infection it is not uncommon to observe wide fluctuations in ALT. Levels may remain normal for months or years only to rise quickly in rare cases, in association with a worsening histological picture.[2] The Italian Association for the Study of the Liver (AISF) some years ago proposed that the time length required to define a PNALT carrier should be 18 months, with a 2-month time lapse between serum assays of ALT levels (a total of 9 assays). This means that a single increase in ALT values above normal in one out of the nine assays excludes patients from the PNALT category.[9]

In the past, HCV carriers with PNALT were excluded from antiviral treatment.[2] More recently, some studies have shown that the rate of virological response in these patients is similar to that observed in patients with high transaminases.[20-23]

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As a result, antiviral treatment is also currently indicated for PNALT carriers, although with some limitations.[24,25] However, at present it is not clear which of these patients are at risk for disease progression and, therefore, whether it is worth treating all of them with antiviral therapy.[24]

The aim of this study was to report the natural history of a group of subjects labeled as PNALT carriers, in accordance with the literature at that time,[26,27] who had undergone liver biopsy in the years 1995-1996 and been followed up for more than 15 years at our centre. In addition, they were divided into two groups according to transaminase levels: those with persistently normal ALT and those with increased ALT values.

The aims were to evaluate:

- How many patients remained PNALT for this long period of time and whether they eventually developed liver disease.
- The stage of liver disease in the patients with elevated serum ALT, and
- What factors could have influenced the progression of liver disease.

MATERIAL AND METHODS

Patients

This study included subjects extrapolated from our previous studies on patients with PNALT,[28,29] who continued to be followed-up in our outpatient clinic for liver diseases. They all had serum transaminase levels persistently within normal limits (i.e. 40 IU/dL) and had undergone liver biopsy in the years 1995/96. Seventy patients met these criteria, but 18 of them were excluded because they were HCV-RNA negative at baseline. The remaining subjects were carefully followed up by monitoring transaminase levels every three months after liver biopsy. To date 7 have dropped out, while 45 continue to be followed up and have been included in this study. In addition, they were divided into two groups according to their serum transaminase levels following liver biopsy: those with PNALT and those with increased ALT levels. The current assessment of patients included a median follow-up of 180 months (range 162-206 months).

Methods

At enrolment to the present study patients underwent a general examination, including the evaluation of body weight, height, body mass index (BMI), blood pressure and heart rate. The main parameters of liver function and lipidemic patterns were evaluated using commercial kits. Markers of hepatitis B virus and qualitative HCV-RNA were also re-assayed and, as previously described,[28,29] patients with a history of alcohol consumption were excluded. To help evaluate their current liver status, diagnostic imaging techniques were also used i.e. ultrasound of the upper abdomen. Furthermore, two current non-invasive markers of liver fibrosis were used i.e. transient elastography and the APRI score, which was compared to the same score calculated at the time of liver biopsy.

Cirrhosis was diagnosed on the basis of the presence and concordance of unequivocal clinical, biochemical and instrumental signs described above.

Ultrasound and color Doppler

Ultrasound of the liver was performed in the morning, after fasting for at least 10 h, by two operators, originally using a real-time Toshiba SSA 270 A apparatus with 3.75 MHz convex and 5 MHz linear probes. As from 2001 a real-time Philips 5000 HDI apparatus was used 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 abilities of the two ultrasound observers (GM, MS) were homogeneous: they had the same professional background, having been trained in this specific field, and both had over a decade of experience.

We considered ultrasound signs of cirrhosis: irregular liver surface associated with signs of portal hypertension (portal vein diameter > 1.2 cm or longitudinal diameter of spleen > 12 cm).[30,31]

Non-invasive markers of liver fibrosis

- **Transient elastography.** TE was assessed by a single certified operator, using TE (FibroScan®; EchoSens, Paris, France). TE provides an assessment of liver stiffness expressed in KPa units as previously described.[32] 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.[33]

- **AST-to-Platelet-Ratio Index (APRI).** Liver fibrosis was also assessed using a well-validated index, the AST 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 value.[34]

Liver biopsy at entry to the study was obtained percutaneously with a Menghini needle and the Histology Activity Index (HAI) evaluated according to Knodell.[35] Genotyping was performed as previously described.[8]

Patients who had increased serum transaminases were treated according to the current therapies and referred to as SVR or NR according to European guidelines.[26]

Arterial hypertension (AH) was diagnosed in accordance with the WHO/ISH criteria.[36] Diabetes Mellitus or Impaired Fasting Glucose (IFG) were diagnosed according to the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus criteria.[37]

Statistical analysis

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 (ρ) correlations were used when appropriate.

To assess which variables measured at baseline were predictive of progression to LC, the univariate Cox proportional hazards (Hr) model was fitted to each variable. All variables with a P-value < 0.05 were subjected to multivariate analysis to assess their value as independent predictors.

$P < 0.05$ was considered significant. Statistical analysis was performed using the SPSS for Windows version 16.0.

RESULTS

As mentioned above, 45 patients (28M, 17F) were included in this study. They continued to be followed up at our Center: every 3 months to evaluate routine hematochemical parameters, and every 12 months for an abdominal ultrasound scan. Patients with US signs of evolution were followed up with a US every 6 months.

Figure 1 shows the evolution of serum ALT and liver disease in the patients during follow-up. Twenty-five patients maintained persistently normal ALT values during the whole follow-up, but two of them developed frank liver cirrhosis. The genotypes of these patients were the following: five had genotype 2, three genotype 3 and seventeen genotype 1b. Among the non-cirrhotic subjects, only two patients (one genotype 2 and one genotype 1b) received antiviral treatment (Peg-IFN + Ribavirin). The decision to treat was primarily based on the determination of the patients to receive treatment, even though there were no signs of progression of liver disease. At the third month of therapy HCV-RNA was negative in both

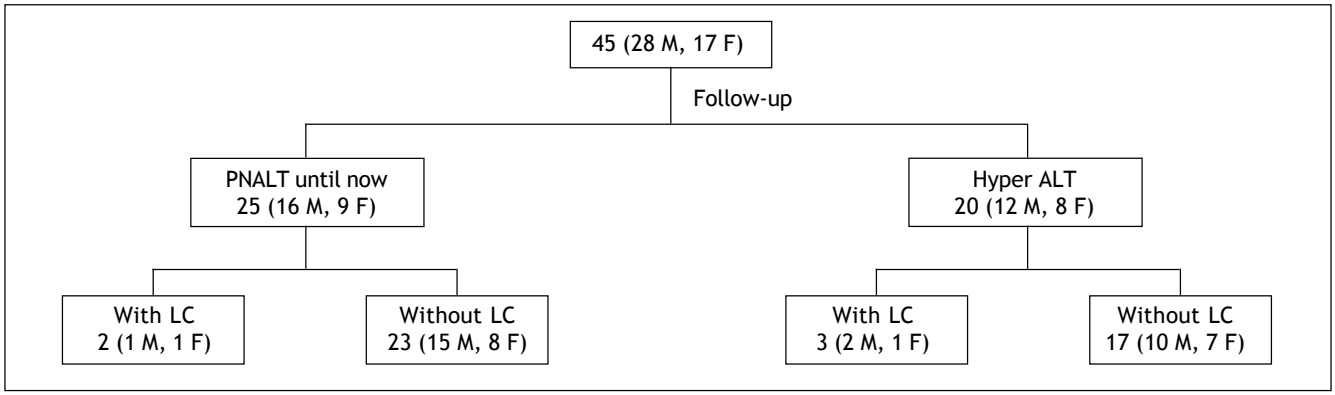

Figure 1. Evolution of the patients studied after 15 years of follow-up.

and at the end they were SVRs. In contrast, twenty patients presented increased serum transaminase levels during the follow-up and three of them developed frank liver cirrhosis. Twelve of these patients underwent current antiviral treatment. Six patients were SVR, while 6 were NR. Among the SVR there were three genotype 1b, two genotype 2 and one genotype 3, whereas all the NR patients had genotype 1b. Two of the three cirrhosis patients were included in the treated patients and one of the two was an SVR.

The remaining 8 patients did not receive treatment because of fluctuating serum ALT levels < 1.5 N in three cases, presence of co-morbidity in 3 cases and due to a brief, temporary increase in ALT in the remaining two, likely related to the assumption of other drugs.

Table 1 compares some baseline demographic, biochemical and histological characteristics of the 45 patients divided according to ALT evolution. This comparison did not reveal any statistically significant differences.

Table 2 shows some demographic, biochemical and histological characteristics at entry to the study of the patients who developed liver cirrhosis, compared to the remaining patients. On univariate analysis, factors associated at baseline with evolution in cirrhosis were: age, hypergammaglobulinemia, arterial hypertension, staging ($P < 0.02$), BMI, HBcAb positivity, grading ($P < 0.05$). On multivariate analysis, staging ($P < 0.04$) and age ($P < 0.05$) were found to be independent predictors of liver cirrhosis.

Table 3 shows the same characteristics quoted in tables 1 and 2 at baseline in patients who had flares of ALT, divided into those with or without LC. Comparing the data, it emerges that on univariate analysis, factors associated with evolution in cirrhosis were: age, arterial hypertension, grading ($P < 0.05$), staging ($P < 0.02$). On multivariate analysis, staging ($P < 0.04$) and age ($P < 0.05$) were found as independent predictors of liver cirrhosis.

At gastroesophageal endoscopy 3/5 LC patients showed variable degrees of esophageal varices.

- **Elastography.** Twenty out of 28 patients underwent TE. 6 overweight patients, one case of Parkinson's disease and one pregnant woman were excluded. Two patients showed values above 8.3 kPa (8.6 in a patient with PNALT and LC and 9.6 in a non-cirrhotic patient with high ALT values) and in only one case was TE above 14 kPa (33.8 in a cirrhotic patient with high ALT values).
- **APRI.** Table 4 shows APRI scores at baseline and at the end of follow-up. Patients evaluated for liver cirrhosis diagnosis showed a significant increase in the score at the end of follow up ($\rho = 0.3$; $P < 0.04$), whereas when they were evaluated for the presence of fibrosis there was no significant difference.

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

	Hyper ALT (n = 20)	PNALT (n = 25)	P <
Sex(M)	12	16	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)	1.31±0.3	1.5 ±0.4	ns
PLT x mmc	375,769±65,156	251,615 ±91,773	ns
Genotype 1	10	7 ns	
HBcAb	20	ns	
Arterial hypertension	20	ns	
IFG or diabetes	00	ns	
Cholesterolemia (g/dL)	176±40	181.7 ±25	ns
Tryglicerides (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 cirrhosis *vs.* those who did not.

	Hyper ALT Without LC (n = 17)	LC (n = 5)	Univariate analysis Hazard ratio (IC 95 %)	P <	Multivariate analysis Hazard ratio (IC 95%)	P<
Sex (M)	25	3	0.8 (0.2-4.6)	Ns	-	-
Age	42.5 ±12.6	52.4 ± 4.1	1.15 (1.1-1.3)	0.02	1.1 (1.05-1.3)	0.05
BMI	25.9 ± 3	29.2 ± 2.2	1.1 (1.03-1.4)	0.05	-	-
Alk. Phos. (U/L)	111.9 ± 49.3	107.4 ± 48.2	1 (0.9-1.0)	Ns	-	-
Gamma-GT (U/L)	23 (8.0-128.0)	32 (16.0-162.0)	1 (0.9-1.1)	Ns	-	-
PT (%)	91.8 ± 8.0	100.0 ± 4.9	1.1 (0.9-1.6)	Ns	-	-
Alb	4.3 ± 0.5	4.0 ± 0.6	0.9 (0.2-4.0)	Ns	-	-
Gamma glob. (g/dL)	1.3 ± 0.3	1.5 ± 0.3	5.7 (1.4-24.)	0.02	-	-
PLT x mmc	316,363 ± 52,000	263,200 ± 13,000	1.1 (0.9-1.2)	Ns	-	-
Genotype 1	13	5	0.4 (0.2-20.0)	ns	-	-
HBcAb	0	2	42 (3.0-90.0)	0.001	-	-
Arterial hypertension	0	3	3	0.02	-	-
IFG or diabetes	2	0	-	-	-	-
Cholesterolemia (g/dL)	187.6 ± 35	172 ± 20	0.1 (0.04-100.0)	Ns	-	-
Triglycerides (mg/dL)	90.7 ± 48	69.2 ± 11	1 (0.9-1.2)	Ns	-	-
HDL-C (g/dL)	53.2 ± 13.0	44 ± 18.0	0.8 (0.5-1.3)	Ns	-	-
APRI	0.28 ± 0.1	0.4 ± 0.1	1.01 (0.6-1.2)	ns	-	-
Grading	4 (0-11)	11 (5-14)	1.2 (1.1-1.5)	0.05	-	-
Staging	0.5 (0-2)	2 (1-3)	2.6 (1.2-5.7)	0.02	2.3 (1.1-9)	0.05

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

	Baseline		Univariate analysis		Multivariate analysis	
	Hyper ALT Without LC (n = 17)	LC (n = 5)	Hazard ratio (IC 95%)	P <	Hazard ratio (IC 95%)	P <
Sex (M)	10	3	0.7 (0.2-4)	ns	-	-
Age	41.1 ± 14.5	52.4 ± 4.1	1.1 (1.05-1.2)	0.05	2 (1.05-7)	0.05
BMI	25.5 ± 3.7	29.2 ± 2.2	1.1 (0.8-1.6)	ns	-	-
Alk. Phos. (U/L)	97.5 (46.0-268.0)	107.4 ± 48.2	1 (0.9-1.0)	ns	-	-
Gamma-GT (U/L)	26 (8.0 ± 162.0)	32 (16.0-162.0)	1 (0.9-1.1)	ns	-	-
PT (%)	94 ± 5	100 ± 4.9	1 (0.9-1.0)	ns	-	-
Alb	4.4 ± 0,5	4.0 ± 0.6	0.6 (0.1-2.6)	ns	-	-
Gamma glob. (g/dL)	1.1 ± 0.3	1.5 ± 0.3	3.8 (0.2-62.0)	ns	-	-
PLT x mmc	429,500 ± 77,000	263,200 ± 13,000	1 (1.0-1.1)	ns	-	-
Genotype 1	3	5	0.3 (0.1-42.0)	ns	-	-
HbcAb	0	2	4.6 (0.8-27.0)	ns	-	-
Arterial hypertension	0	3	6.4 (1.1-38.8)	0.05	-	-
IFG or diabetes	0	0	-	-	-	-
Cholesterolemia (g/dL)	176.0 ± 40.1	172.0 ± 20.2	0.9 (0.9-1.1)	ns	-	-
Triglycerides (mg/dL)	89.7 ± 40.0	69.2 ± 11.0	1 (0.9-1.2)	ns	-	-
HDL-C (g/dL)	52.2 ± 12.3	44.0 ± 18.1	0.8 (0.5-1.3)	ns	-	-
APRI	0.3 ± 0.1	0.4 ± 0.1	1.1 (0.8-1.3)	ns	-	-
Grading	5 (0-14)	11 (5-14)	1.2 (1.1-1.8)	0.05	-	-
Staging	1 (0-3)	2 (1-3)	1.8 (1.2-3.7)	0.02	2.8 (1.1-9)	0.04

Table 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	ρ = 0.15; P = ns
0.5-1.5	Unclassified to significant fibrosis	3	1	
> 1.5	Presence of significant fibrosis	0	4	
< 1	Absence of cirrhosis	28	24	ρ = 0.31; P < 0.04
1-2	Unclassified to cirrhosis	0	1	
> 2	Presence of cirrhosis	0	4	

ρ: Spearman's rank correlations.

DISCUSSION

In our study population 5/45 HCV-RNA positive patients developed frank liver cirrhosis. This result is not so relevant, as it is lower than the figures already reported in various studies in the literature[12,14] and especially in some Italian studies.[13,15] Nevertheless it is higher than in other Italian reports, where the progression to liver cirrhosis was reported to be slow or absent.[18,38] In any case, it confirms that PNALT patients need to be monitored in the same way as patients presenting high ALT levels, since it is difficult to distinguish in which of the patients presenting with PNALT liver disease may progress. The prevalence of subjects with normal liver at biopsy (true "carriers") is lower than 20% of all PNALT patients.[2,6-10] In most cases there are variable degrees of liver damage, fibrosis is usually mild or absent[10] and histology is generally less severe than in patients with high or fluctuating serum ALT levels.[2,7,10,11] Recent studies, however, have shown more severe liver damage (fibrosis ≥ F2) in at least 20% of cases and liver cirrhosis in 3-5% of patients. There have also been rare cases of hepatocellular carcinoma in patients with normal ALT,[15,16] even with a histologically normal liver structure.[17]

Although limited by the small number of patients, our data would appear to confirm that subjects older in age and presenting a higher degree of liver fibrosis at baseline are candidates for liver disease progression. Other factors which, 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, or even only an association with anti-HBc positivity. Therefore, it is of paramount importance to monitor this particular category of patients and to correct any eventual metabolic disturbances (i.e. insulin resistance) to prevent the development of a metabolic syndrome, which will worsen the evolution of liver disease.

Similar data were reported by Persico M, *et al.* who studied the natural history at ten years of a group of 24 patients with PNALT, compared with a group of 40 patients with high levels of transaminases, using liver biopsy at baseline and after 5 and 10 years. They did not report any significant histological differences in the three liver biopsies in the PNALT patients, thus suggesting that cirrhosis progression is low or absent in these patients. 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 patients, in common with other reports in the literature, can be justified by the fact that in order to obtain accurate information a liver biopsy should be performed at both the beginning and the end of the study, which is not justifiable ethically, or only in controlled studies dedicated to this purpose. One limitation of our study is certainly that a second liver biopsy was not performed after all these years, although as a surrogate we used liver function tests, ultrasound, elastography and clinical examination to avoid resorting to invasive techniques in the majority of the patients, in whom disease did not progress. However, it is mandatory to continue monitoring these patients, because as we now know from studies of the natural history of HCV disease, it may take decades and sometimes longer before HCV infection progresses to cirrhosis. Consequently, patients with normal livers today could later develop evidence of hepatic impairment. These considerations, together with the evidence that combination therapy is equally effective in subjects with normal ALT, once again indicate that the two groups of patients are not substantially different, consequently, the same criteria for follow-

up and treatment recommended for patients with elevated ALT should be applied to subjects with normal ALT.[39]

Carriers of HCV with PNALT have traditionally been excluded from antiviral treatment, both in trials and in clinical practice.[2] A first major therapeutic development occurred with the use of IFN-ribavirin combination therapy: virologic response rates were obtained that did not differ from those seen in patients with elevated transaminases, as evidenced by a study by Mangia, *et al.* and later confirmed by other works.[20-23] In Italy the treatment of hepatitis C with pegylated interferon alfa-2a and ribavirin is currently allowed in HCV carriers independent of their transaminase levels.[40] However, in view of the economic costs, it is essential to carefully select the patients to be referred for treatment. Fundamental criteria for defining optimal treatment protocols may be a patient's age, his/her motivations, the possibility of eradication (viral genotype), life expectancy, duration of disease, the presence of co-factors of liver disease, adherence to treatment, contraindications, considerations about infectiveness (if the subject is promiscuous or has a stable partner), type of employment (potential infection of others). On the contrary, in patients where the cost-benefit 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, depending on the severity of liver histology. Therapy should thus be reserved for only patients with a high grade of fibrosis (> F2) subjects with or without moderate fibrosis need to be closely monitored. Finally, in subjects aged over 60-65 years and with a long duration of disease, it would seem reasonable to carry out a regular clinical follow up, thus avoiding both liver biopsy and treatment.

CONCLUSION

In conclusion, our data show that almost half of HCV-RNA positive subjects defined as PNALT, according to the definition of The Italian Association for the Study of the Liver, i.e. 18 months of follow-up with at least 9 determinations, will have flares of ALT in the long term follow-up but few of them will develop LC. In the other half, serum transaminases will remain within normal limits, but in this group the risk of developing LC, although lower, is also present. As well as the transaminase levels, other factors related to age at the start of infection, grading and staging at histology and the concurrence of some metabolic factors, seem to play an important role in the progression of liver disease. Therefore, as well as ensuring a close clinical, biochemical and instrumental follow-up of liver disease, it is our duty to at least address these modifiable factors, which could make the difference in preventing the progression of liver disease.

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