

Correlations Between METAVIR Staging, the Viral Load and Alanine Aminotransferase Levels in Patients with Chronic Hepatitis C, Before and after 12 Weeks of Antiviral Treatment

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The World Health Organization (WHO) estimates that 170 million individuals worldwide are infected with hepatitis C virus (HCV). The aim of study was to evaluate possible correlations between METAVIR staging, the viral loads and alanine aminotransferase (ALT) levels in patients with chronic hepatitis C, before and after 12 weeks of treatment with PegInterferon $\alpha 2a/\alpha 2b$ and Ribavirin. 93 consecutive patients with hepatitis C, were included in this study. The parameters followed were age, sex, Metavir histologic staging at the start of the treatment, hepatitis C virus ribonucleic acid (HCV-RNA) and ALT at start and after 12 weeks of treatment, as well as alcohol intake. The study group was composed by 58 women and 35 men, with a mean age of 44.47 ± 11.17 years. At the beginning of the treatment, the mean value of HCV-RNA was 1327163 ± 145001 UI/mL, with a $CI_{95\%} = 1327163 \pm 287986.36$ UI/mL, and the mean value for ALT was 112.7124 ± 68.7403 U/l. After 12 weeks of therapy, significant differences were found concerning both HCV-RNA and ALT values. HCV-RNA lowered to a mean value of 457.3978 ± 402.653 ($p < 0.0001$), while ALT lowered to a mean value of 37.90 ± 16.43 U/l ($p < 0.0001$). Neither the initial HCV-RNA nor ALT values had any correlations with the METAVIR histological staging of the fibrosis. Initial ALT was higher in those with alcohol intake ($p = 0.000048$). The ALT values instead, after 3 months of treatment, decreased significantly ($p < 0.0001$), did correlate with the METAVIR groups ($p = 0.0019$). The evolutionary stage of fibrosis didn't correlate with gender of the patients or initial values of the HCV-RNA or ALT. There was no correlation between the age/gender of the patients and the determined METAVIR score.

Keywords: METAVIR staging, HCV RNA, ALT, PegInterferon, Ribavirin

The World Health Organization (WHO) estimates 170 million individuals worldwide are infected with hepatitis C virus (HCV). However, the prevalence of HCV infection varies throughout the world. For example, in 2000, Frank et al reported that Egypt has the highest number of reported infections, largely attributed to the use of contaminated parenteral antischistosomal therapy [1]. This has led to a mean prevalence of HCV antibodies in persons in Egypt of 22%. RNA-dependent RNA polymerase, an enzyme critical in HCV replication, lacks proofreading capabilities and generates a large number of mutant viruses known as quasispecies [2,3]. These represent minor molecular variations with only 1-2% nucleotide heterogeneity. HCV quasispecies pose a major challenge to immune-mediated control of HCV and may explain the variable clinical course and the difficulties in vaccine development. In most infected people, viremia persists and is accompanied by variable degrees of hepatic inflammation and fibrosis. Findings from studies suggest at least 50% of hepatocytes may be infected with HCV in patients with chronic hepatitis C [4,5].

Histological Findings: Lymphocytic infiltration, moderate degrees of inflammation and necrosis, and portal or bridging fibrosis are noted. Regenerative nodules are seen in patients with cirrhosis. Some patients also may

have findings indicative of HCC. Most pathologists give separate measurements of disease activity (grade) and fibrosis (stage). Many trials use the Ishak (6-point scale) and Knodell histological activity index (18-point score); both are useful for assessing improvements in histology findings in studies but are impractical for clinical use because of interobserver disagreement. The METAVIR score was developed by the French METAVIR Cooperative Study Group and reported by Bedossa and Poinard in 1996. The METAVIR score helps interpret a liver biopsy. The fibrosis is graded on a 5-point scale from 0 to 4. The activity, which is the amount of inflammation (specifically, the intensity of necro-inflammatory lesions), is graded on a 4-point scale from A0 to A3. [6,7] Fibrosis score: F0 = no fibrosis; F1 = portal fibrosis without septa; F2 = portal fibrosis with few septa; F3 = numerous septa without cirrhosis; F4 = cirrhosis. Activity score: A0 = no activity; A1 = mild activity; A2 = moderate activity; A3 = severe activity

Aim of study was to evaluate possible correlations between METAVIR staging, the viral loads and ALT levels in patients with chronic hepatitis C, before and after 12 weeks of treatment with standard doses of either Peginterferon $\alpha 2a$ (180 μ g/week)/or $\alpha 2b$ (1.5 μ g/kgc/week) plus Ribavirin in standard doses (1g/day under 75kg or 1.2g/day at more than 75kg body weight).

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Experimental part

Material and methods

93 consecutive patients with hepatitis C, who were treated in the 2nd Department of Internal Medicine at the Emergency County Hospital Mures in the last three years, were included in this study. The parameters followed, were age, sex, Metavir histological staging at the start of treatment, quantitative HCV-RNA at start and after 12 weeks of treatment, and the levels of ALT at the same points. We also made correlations with alcohol intake. The methods used for evaluation of the parameters were: Real-time-PCR for HCV RNA, with detection limit of 15 IU/mL, standardized kinetic method without pyridoxal phosphate for ALT, with detection limit of 5 U/L. METAVIR histological staging was performed by liver biopsy, using 2-2.5 cm of liver tissue, fixed in formalin, then stained with hematoxylin-eosin or using special stains. The statistical methods used in order to assess possible correlations between these parameters in our study were: descriptive statistics, F-test two samples for variances, Student t-test, and simple linear correlation. All tests and graphics were computed using Microsoft Office Excel 2010, and Statsoft Statistica for Windows 8.0.

Results and discussions

The studied group was composed by 58 women and 35 men, with a mean age of 44.47 ± 11.17 years. Using the METAVIR score the patients were distributed in several groups. The majority of patients were in stages A2F2 and A2F3 according to METAVIR staging (fig. 1).

Descriptive statistics found a mean value of 1327163 UI/ml, with a standard error of ± 145001 UI/mL ($CI_{95\%} = 1327163 \pm 287986.36$ UI/mL) for HCV-RNA (lower detectable value being 15 IU/mL) and of 112.7124 ± 68.74033 U/L for ALT (normal standard value 40 U/L), before treatment. Comparing the values before treatment with those detected after 12 weeks of antiviral therapy, significant differences were found concerning both HCV-RNA and ALT values. HCV-RNA lowered to a mean value of

457.3978 ± 402.653 ($p < 0.0001$), in 80 cases being undetectable, while ALT fell to a mean value of 37.90 ± 16.43 U/L ($p < 0.0001$), in 54 cases being under 40 U/L.

After the correlations we made, the most important results were:

- No significant correlation could be made between the age of the patients and the initial HCV-RNA values ($R^2 = 0.0019$).

- No significant differences were found between gender and HCV RNA values ($p = 0.416$).

- No statistical significant correlation could be established between the age of the patients and initial ALT values ($R^2 = 0.0011$).

- In male patients the ALT values were significantly increased ($p = 0.019$). This result has to be interpreted in corroboration with HCV-RNA impact on ALT.

- There was no correlation between the initial values of ALT and HCV-RNA ($R^2 = 0.0164$) (fig. 2).

- As a next step we examined the alcohol consumption, as the possible secondary factor. The incidence of an over 20 mg/day alcohol ingestion was: in males: 45.71%, in females: 8.77%

- The initial ALT values were significantly higher in those with an over 20 mg /day alcohol consumption ($p = 0.00048$) (fig. 3).

- After 3 months of treatment and no alcohol ingestion, the ALT values decreased (fig. 4).

Using METAVIR staging, we determined the correlations between the grade of fibrosis and HCV-RNA and ALT respectively, at start and after 12 weeks of treatment.

- The HCV-RNA mean values, before and after the 12 week treatment, hadn't show any significant variability with the METAVIR histological staging of the fibrosis ($p_{\text{initial RNA}} = 0.679$; $p_{\text{3month RNA}} = 0.99$) (fig. 5)

- The variance of the mean ALT values wasn't significant at the beginning of the study, but after 3 months of treatment, the variance proved to be highly significant ($p_{\text{initial ALT}} = 0.503$; $p_{\text{3month ALT}} = 0.0019$) (fig. 6)

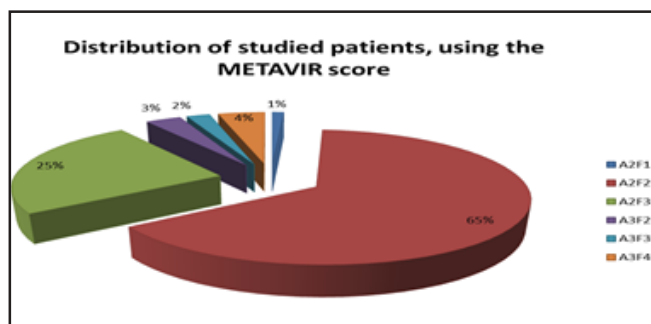


Fig. 1. The numeric distribution of the patients using the METAVIR score

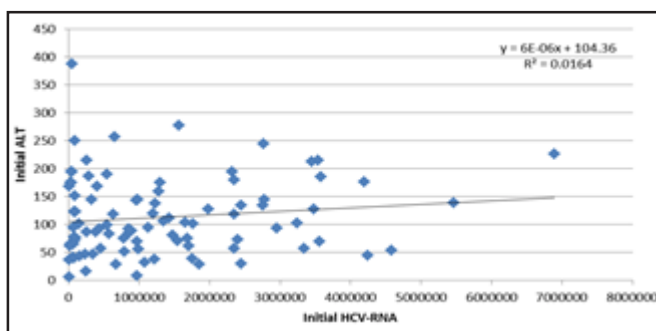


Fig. 2. Initial HCV-RNA values correlated with initial ALT values

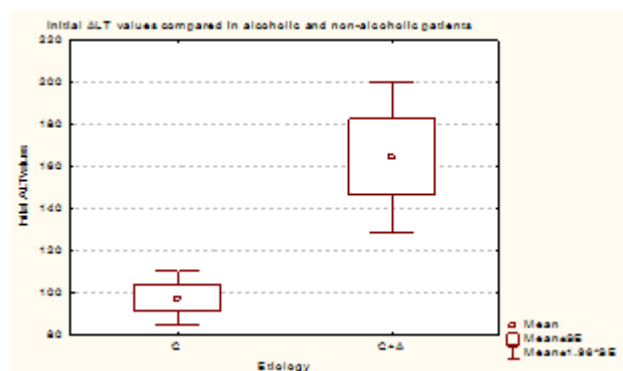


Fig. 3. Initial ALT values in alcoholic and non-alcoholic patients

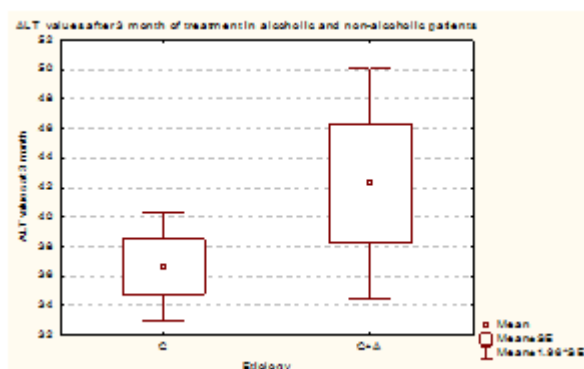


Fig. 4. ALT values after 3 month of treatment in alcoholic (most of them have abandoned the alcohol consumption) and non-alcoholic patients.

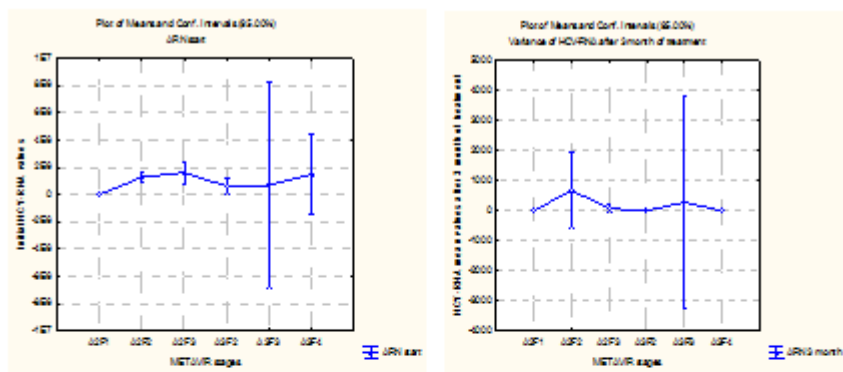


Fig. 5. Variance of HCV-RNA mean values before and after 3 months of treatment

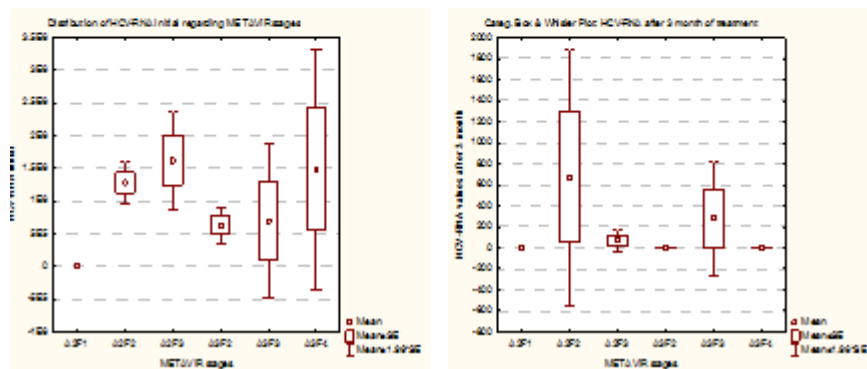
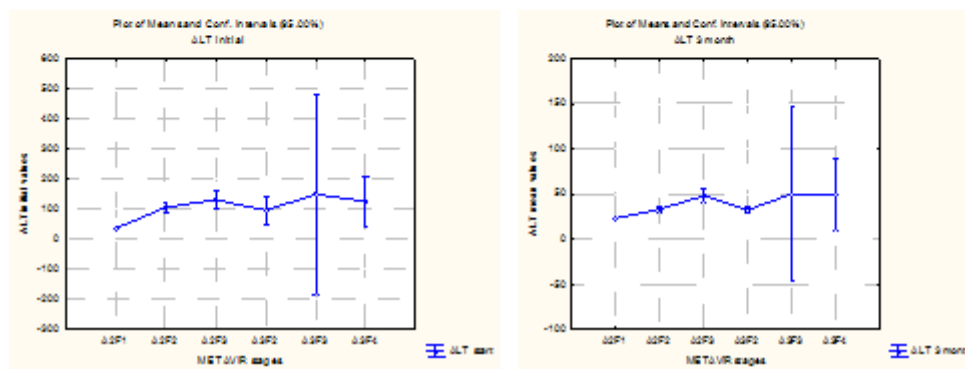
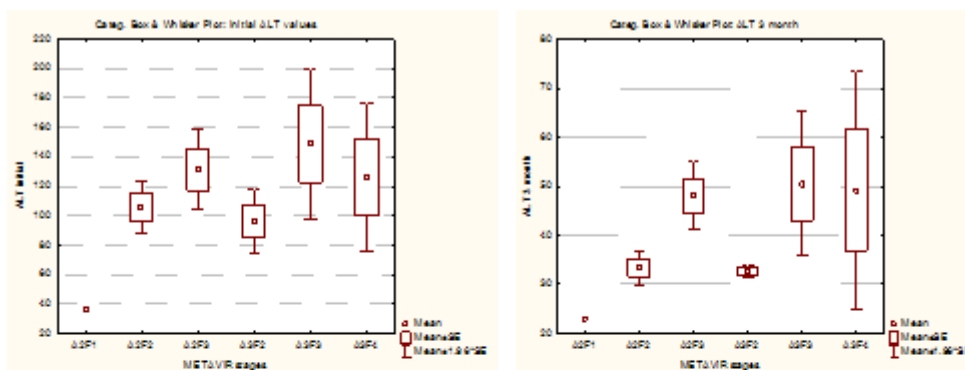


Fig. 6. Variance of ALT mean values before and after 3 month of treatment



-After 12 weeks of treatment, the values of HCV-RNA were significantly decreasing ($p < 0.0001$) (fig. 7)

-The ALT values instead, after 3 months of treatment, decreased significantly ($p < 0.0001$) (fig. 8), and the variance of the ALT mean values correlate with the METAVIR groups ($p = 0.0019$) (fig. 11)

-No correlation could be made between the initial values of HCV-RNA and ALT ($R = 0.13$). After 3 months of treatment, we managed to find a moderate positive correlation between these values ($R = 0.26$, correlations are significant at $p < .05000$) (fig. 9 and 10).

Treatment with Peginterferon plus ribavirin, for 48 weeks is recommended for patients infected with HCV genotype

1, which represents the most common variant in Europe, and the majority in our country. Early virological response (EVR) analyzed after 12 weeks of treatment, defined as decrease of viral load ≥ 2 log or undetectable HCV RNA. [8] Comparing our results with other studies, we reached comparable results, showing no correlation between the grade of fibrosis and EVR. The time to the first undetectable HCV RNA level is associated with the probability of virologic relapse after the end of treatment. EVR at 12 weeks is a good predictor for sustained virologic response. Thus, HCV RNA kinetics has strong predictive value [9,10].

Lock et al, in a retrospective study on 126 pts with chronic viral hepatitis C concluded that there was no

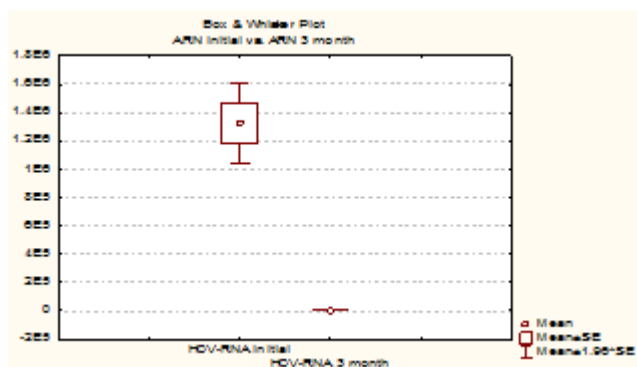


Fig. 7. Decrease of the HCV-RNA values after 3 month of treatment

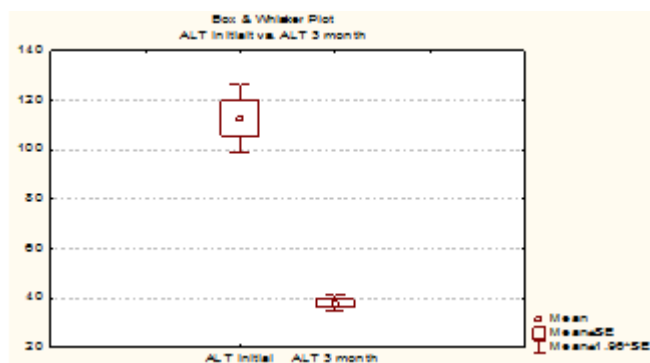


Fig. 8. Decrease of the ALT values after 3 month of treatment (and no alcohol ingestion)

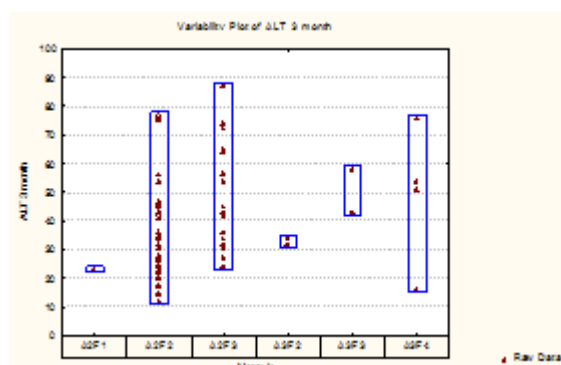


Fig. 11. Variability of ALT after 3 month of treatment vs. METAVIR groups

| Fixed Effect Test for ALT 3 month Restricted Maximum Likelihood (REML) Type V decomposition | | | | |
|---|------------|------------|----------|----------|
| Effect | Num. DF | Den. DF | F | p |
| Metavir | 5 | 87 | 4.170114 | 0.001918 |

association between inflammation or fibrosis and serum viral load. Inflammatory grading showed a moderate but significant correlation with ALT ($p \leq 0.001$), whereas staging of fibrosis did not correlate with ALT. There was no association between grading or staging and serum viral load. Aminotransferases as *surrogate markers* reflect more or less the histological inflammatory activity but do not allow any estimation of the extent of fibrosis. Some patients may have a high inflammatory activity with low aminotransferases or high aminotransferases with low inflammatory activity. Virological parameters such as HCV genotype or viral load do not allow an estimation of histological findings. If prior to treatment of chronic hepatitis C liver biopsy is omitted and the decision for treatment depends solely on surrogate markers, considerable misjudgment of the actual status of liver inflammation or fibrosis may result [11].

Ticehurst et al, analyzed the baseline characteristics of 2472 HCV infected patients and concluded that increasing

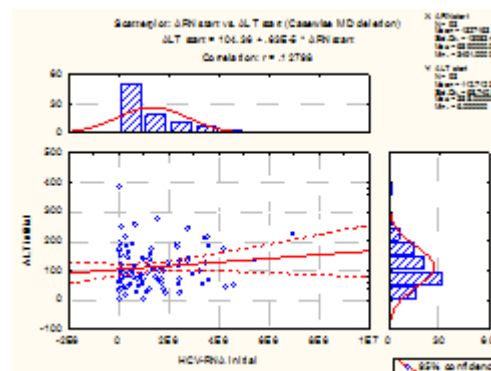


Fig. 9. Correlation between initial ALT values and HCV-RNA initial values

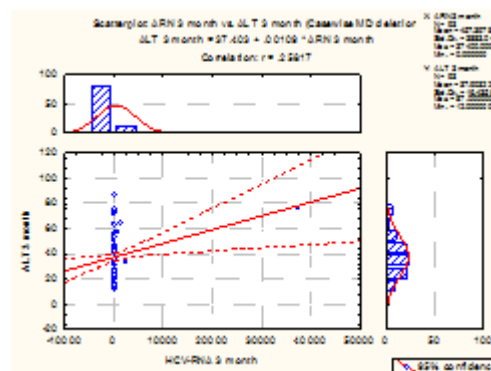


Fig. 10. Correlation between ALT values and HCV-RNA values after 3 month of treatment

HCV RNA was correlated with with higher fibrosis and necrosis- inflammation scores [12].

Factors associated with a smaller decrease in the HCV RNA level are in a study made by Hoofnagle et al, African American race, higher initial HCV RNA level, more severe hepatic fibrosis, and higher body weight [13-17].

Akkaya et al, in a study on 36 pts, also concluded that ALT levels correlate with duration of HCV infection, and histologic activity index, reflecting alterations in the liver tissue [1].

In our study we could not estimate the duration of the infection, this could be one reason for different results comparing with the last three mentioned studies.

Conclusions

The evolutionary stage of fibrosis didn't correlate with gender of the patients or initial values of the HCV-RNA or ALT. We found no correlation between the age of the patients and the determined METAVIR score.

Initial values of ALT are positively correlated with gender, showing higher values in men. We believe that these results are given by increased alcohol consumption in this group.

The positive correlation between ALT after 3 months, and the METAVIR score, shows us a possible better response to the treatment in patients with lower stage of fibrosis. Despite the increased initial values of ALT in all groups, ALT decreases more in those with less severe fibrosis.

Correlation between HCV-RNA and ALT could not be considered significant, because of the HCV-RNA undetectable value measured in numerous cases after 3 month of treatment. The absence of correlation between the initial values of HCV-ARN, and ALT, suggests that the degree of the fibrosis, and cellular destruction caused by viral infection is not directly related to viral replication, and the evolution of fibrosis also involves other factors and mechanisms.

Abbreviations

ALT= alanine aminotransferase

HCV RNA= hepatitis C virus ribonucleic acid

HCV= hepatitis C virus

HCC= hepatocellular carcinoma

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