# Clinical outcome in a cohort of HIV/HCV coinfected patients. HCV RNA presence associated to higher GGT levels.

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## Introduction - Objectives

The 90% of haemophilic men were exposed to Hepatitis C Virus (HCV) through clotting factor concentrates prior to viral inactivation procedures. HCV is the most common cause of chronic liver disease and the leading cause of death in individuals with haemophilia. It is well recognized that older age and longer duration of HCV infection, high serum HCV-RNA levels, HCV genotype, alcohol abuse, insulin resistance, antiretroviral drug modality and Human Immunodeficiency Virus (HIV) ,accelerates the progression of chronic liver disease to liver failure and death in co-infected patients. Non-invasive markers of hepatic fibrosis e.g: serum gamma glutamyltransferase (γ-GT) aspartate aminotransferase (AST), alanine aminotransferase (ALT), platelet count and combinations have been increasingly used to predict liver disease. It has been well demostrated that IL28B polymorphisms influence both, the rate of spontaneous HCV clearance and the response to interferon α (IFN α)-based therapy. This observation has been reproduced in HIV-co-infected individuals. However, the influence of IL28B polymorphisms on hepatitis C outcomes, including early viral kinetics on therapy, progression of liver fibrosis, or HCV plasma viral load has been assessed by several groups with discordant results.
**Aims of the study:** To look for clinical, immunological and virological markers that could be useful to evaluate the clinical progression in a group of 38 HIV/HCV coinfected hemophilic patients.
To determine IL28b polymorphisms in our population in order to find an association with their clinical evolution.

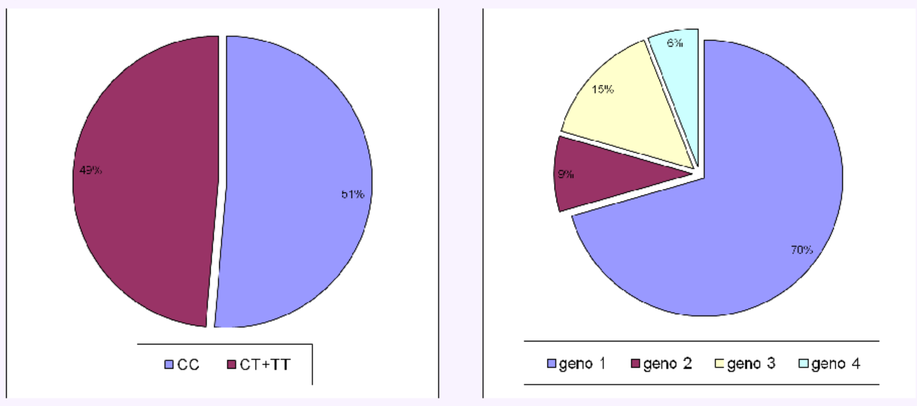
## Methods

**Study population and Methods:** Different parameters like age, HIV and HCV infections presence and viral loads, hepatic enzymes (AST, ALT, alkaline phosphatase (ALP) and γ-GT), CD4/CD8 T cell counts, and platelet counts were evaluated as surrogate clinical progression markers. BMI (Body Mass Index), HOMA (Homeostasis Model Assistment index), APRI (AST-to- platelet ratio index) and FORNS indexes were also calculated. The rs12979860 genotype of IL 28b was determined by polymerase chain reaction (DNA specimens was collected from peripheral blood mononuclear cells (PBMC).The results were reported as two categories: CC, and CT+TT. HCV and IL28b genotype distributions are shown in figure below. Clinical parameters and HIV/HCV status were collected from medical records from 2008 to 2012. See the table below for the clinical, biochemical, and virological characteristics of the patients.
**Statistical Analyses :** Student t test or Mann Whitney were used to compare quantitative results and Chi square or Fisher test to analyze qualitative parameters of the study population.

| Patient Profiles / No.Patientes:38 | Mean | Median | Range |
|---|---|---|---|
| Age | 40.25 | 39 | 24-69 |
| BMI | 26.19 | 25.8 | 21-32,20 |
| Platelet count $10^3$/ul (NV: 150-400) | 196.1 | 198 | 80-292 |
| Ferritin level ng/ml (NV: 20-400) | 613.1 | 306.5 | 122-3546 |
| α-Fetoprotein level ng/ml (NV: to 10) | 3.92 | 2.35 | 1,3-19,4 |
| AST level UI/L (NV: to 38) | 49.53 | 43 | 17-174 |
| ALT level UI/L (NV: to 41) | 58.81 | 49 | 14-168 |
| Cholesterol level mg/dl (NV: 150-220) | 169.8 | 170 | 98-238 |
| γ-GT UI/L (NV: 11-50) | 100.8 | 62.75 | 22-315 |
| ALP level UI/L (NV: 65-300) | 280.4 | 269.5 | 167-519 |
| CD4 count cells/ul | 452.5 | 399 | 47-1384 |
| HIV VL log UI/ml | 2.49 | 1.69 | 1,69-5,32 |
| HCV VL log UI/ml | 4.12 | 5.75 | 0-6,9 |
| APRI Score | 0.81 | 0.54 | 0,16-4,72 |
| FORNS Score | 5.10 | 4.70 | 1,94-8,16 |

**Table 1:** BMI: body mass index; AST: aspartate aminotransferase; ALT, alanine aminotransferase; γ-GT: gamma glutamyltransferase; ALP: alkaline phosphatase, APRI: AST-to- platelet ratio index; HOMA: Homeostasis Model Assistment index; NV: Normal Value; VL: Viral Load.
**Additional considerations:** Alcohol intake in our population was considered between normal values. Two times the upper limit for normal range were considered abnormal γ-GT values. For AST and ALT, the abnormal values were 3 times their upper normal limit.

Figure 1




## Results

1. We observed no statistically significant differences for markers between genotypes CC and CT+TT groups (p>0.05).
2. A significant number of patients showed elevated γ-GT levels. Thirteen of 29 patients (45%) showed γGT values ≥ 2XNormal γGT with a median reaching 175UI/l , mean: 199±79 UI/l (p<0,0001). This group of patients showed significantly higher HCV viral loads. See Table

| | γGTx2 (n=13) | | | γGT N (n=16) | | | |
|---|---|---|---|---|---|---|---|
| | mean | median | range | mean | median | range | p |
| γGT value (UI/l) | 199 | 175 | 105-315 | 33 | 31 | 22-50 | **<0.0001** |
| HCV VL (log UI/ml) | 6.021 | 6.12 | 3.8-6.9 | 3.17 | 3.89 | 0-6.4 | 0.0048 |
| FORNS | 5.8 | 5.56 | 4.17-8.21 | 4.2 | 4.25 | 1.94-8.16 | 0.005 |

3. Abnormal γGT values were associated to HCV Viral loads in a logistic regression model also controlled for other parameters.

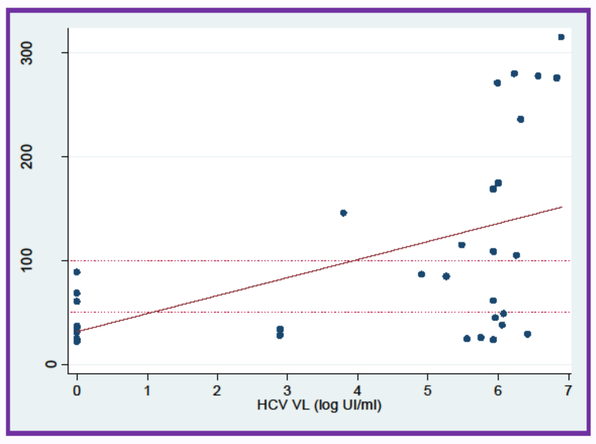
**Logistic regression**

| | | | | | | |
|---|---|---|---|---|---|---|
| Log likelihood = -6.0336814 | | | | Number of obs | = | 27 |
| | | | | LR chi2(7) | = | 25,03 |
| | | | | Prob > chi2 | = | 0,0008 |
| | | | | Pseudo R2 | = | 0,6747 |
| αGTP x 2 | Coef. | Std. Err. | z | P>z | [95% Conf. | Interval] |
| HCV VL | 1,6358 | 0,772 | 2,12 | 0,034 | 0,121176 | 3,150424 |
| CD4 | 0,0007 | 0,00356 | 0,19 | 0,85 | -0,0063075 | 0,007654 |
| AST | -0,03 | 0,252962 | -0,12 | 0,905 | -0,526139 | 0,465455 |
| ALT | 0,2531 | 0,144521 | 1,75 | 0,08 | -0,0301801 | 0,53633 |
| plaq | -0,1 | 0,071495 | -1,4 | 0,16 | -0,2405006 | 0,039753 |
| ALP | 0,0582 | 0,026557 | 2,19 | 0,029 | 0,0061174 | 0,110217 |
| _cons | -9,856 | 10,97825 | -0,9 | 0,369 | -31,3727 | 11,66126 |

4. We also observed a positive correlation between γ-GT levels and HCV VL (r=0.55; p=0.003; r2=0.30)
5. γ-GT levels were not associated with IL28 alelle polymorphisms .

| αGTP | Coef. | Std. Err. | t | P>t | [95% Conf.Interval] | |
|---|---|---|---|---|---|---|
| HCV VL | 17,35162 | 5.299931 | 3.27 | 0,003 | 6,542339 | 28,1609 |
| _cons | 31,7754 | 26.01989 | 1.22 | 0,231 | -21,29251 | 84,84331 |

6. Among the group with non detectable (ND) HCV RNA in plasma (spontaneous or therapy-induced clearance) (n=11), higher platelet counts were observed (p=0.04) in parallel with lower γ-GT (p=0.03), lower FORNS index (p=0.008) and greater CD4+ cell counts (p=0.03).




7. When considering the group with ND HIV viral load, CD4 were significantly increased (p=0.004) but also, higher γ-GT levels were observed (133 vs 66 UI/l; p=0.02). Analysis of the data inside this group showed that patients with detectable HCV viral loads had significantly higher γ-GT levels (175 vs 48 UI/l, p=0.01) and FORNS index was also increased but no statistically significant (5.85 vs 4.5, p=0.06), than patients with ND HCV loads.
8. 11/11 (100%) of the patients with Sustained Viral Response (SVR) or spontaneous clearance had normal γ-GT values (median=34 UI/ml). Seven out of 8 (87,5%) from the non responder patients showed abnormal γ-GT values (median=220 UI/ml).Taken together with the fact that only 45% of the SVR had to CC-IL28 genotype, it appears in our cohort, γ-GT levels showed stronger association for treatment response than IL28 polymorphisms. More patients should be studied to confirm this observation.
9. Considering metabolic disorders and insuline resistance , these conditions were not related to abnormal γ-GT values.

## Observations

Serum γ-GT level is frequently increased in patients with chronic HCV with prevalence ranging between 38% and 54%. The increased γ-GT level is relevant to clinical practice as a host-related factor of poor response to treatment. Furthermore , this enzyme seemed to be useful as an indirect marker of liver compromise. The mechanisms responsible for γ-GT increase are uncertains. First, excessive alcohol intake (40 g per day for men) but also many genetic and environmental factors may be involved, and the true meaning of the γ-GT alteration frequently remains unclear. In addition , levels with normal ALP are frequently observed in patients with chronic hepatitis C, and more often than in other forms of viral hepatitis. Moreover, increased γ-GT may reflect an association between HCV infection and steatosis and may indicate the cholestasis that often accompanies chronic HCV infection at liver histology. Also, increased levels of γ-GT have been consistently found to be associated in the general population with diabetes and with other metabolic risk factors.
In our study the γ-GT increase was only significantly associated to the presence of the HCV RNA in plasma samples. The liver histopathological changes due to viral presence could result in an overflow of γ-GT into the bloodstream. This observation deserves further analysis on the mechanisms involved.

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## Aknowledgements

This study was supported by grant from SECYT PICT 0393-2008. We gratefully acknowledge **Fundación René Baron** and **Pfizer** for financial support for travel and meeting registration. We are indebted to **Fundación Argentina de la Hemofilia** (health professionals, administrative staff and technicians) and the patients with hemophilia for their financial/scientific support and generous cooperation.