

COMPARATIVE ANALYSIS OF HCV RNA LEVELS IN LIVER, PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC) AND SERUM OF PATIENTS WITH HIV-HCV COINFECTION VERSUS PATIENTS WITH HCV MONOINFECTION.

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ABSTRACT

Background. Aim of the study was to analyze HCV RNA levels in serum, liver and PBMC of patients with high aminotransferase levels and HCV-HIV coinfection with respect to patients with HCV monoinfection.

Methods. Two groups of patients were examined: i) 45 patients with HCV-HIV coinfection receiving HAART (group A), ii) 77 patients with HCV monoinfection (group B). Patients in both groups were not previously submitted to interferon treatment. HCV RNA in serum, liver biopsy and PBMC samples was quantified. In addition, HCV genotype was determined and liver histology was evaluated.

Results. HCV RNA serum levels were significantly higher in group A in comparison to group B [14,940,000, range 252,000-118,900,000 vs 6,104,000, range: 199,000-74,620,000 genome equivalents (GE)/ml, respectively]. In contrast, liver and PBMC RNA levels were significantly lower in group A compared to group B. In fact, median HCV RNA levels in liver and PBMC were 16,666 (range: 469-131,578) vs 78,125 (range: 250-1,250,000) GE/ μ g total RNA and 937 (range: 0-8,333) vs 3,958 (range: 0-62,500) GE/ μ g total RNA, respectively. No difference in the distribution of HCV genotype or in histologic findings were observed between the 2 groups of patients.

Conclusions. A dissociation between the reported higher serum level and the observed lower liver and PBMC HCV RNA levels in patients with HIV-HCV coinfection with respect to patients with HCV monoinfection was found. The mechanisms responsible for the dissociation remain to be further investigated.

INTRODUCTION

After the introduction of the highly active antiretroviral therapy (HAART) the life expectancy of patients with HIV infection has improved. However, morbidity and mortality rates of liver diseases are still substantial problems in HIV-infected individuals (1).

Higher levels of HCV RNA in serum of patients coinfecting with HIV, compared to patients with HCV mono-infection, were reported (2). Although the pathogenetic basis for this observation was not clarified, HIV-induced immune deficiency was considered a potential factor (3). On the other hand, a correlation between serum HCV RNA levels and CD4⁺ T cell counts was not confirmed (4). In HIV-positive patients treated with HAART controversial data showing no significant changes (5), increased (6) or decreased (7) HCV RNA serum levels were reported. On the other hand, a direct relationship between serum HCV RNA levels and severity of liver lesions is not universally accepted (8-9).

In several studies intrahepatic HCV RNA levels in patients with chronic liver disease or in transplanted patients (9-10) were examined, whereas only scant data are available for HIV-HCV coinfecting patients (11).

The aim of this study was to analyze HCV RNA levels in serum, liver and PBMC of patients with HIV-HCV coinfection in comparison to patients with HCV mono-infection.

MATERIALS AND METHODS

Patients. 45 HAART-treated HIV-positive patients (group A) and 77 HIV-negative patients (group B) were enrolled between March 1998 and December 1999. Inclusion criteria were: presence of anti-HCV antibodies, serum HCV RNA positivity, availability of liver biopsy specimens, no previous treatment with interferon or interferon and ribavirin, high aminotransferase levels for at least 6 months.

Specimens. The histological activity index (HAI) and the degree of fibrosis were evaluated in liver specimens.

Blood specimens were obtained the same day of liver biopsy. Peripheral blood mononuclear cells (PBMC) samples were obtained for 41/45 patients of group A, for 62/77 patients of group B, while serum samples were available for all patients. Finally, the aminotrasferase levels were determined for each patient the same day of liver biopsy.

HCV and HIV RNA quantification. HCV RNA in serum was quantified using the Quantiplex HCV RNA 2.0 assay (Bayer, Eragny, France), while HCV RNA was quantified in liver and PBMC by a modified RT-PCR method (12). HIV RNA in plasma was quantified using the Quantiplex HIV-1 RNA 3.0 assay (Bayer).

Statistical analysis. Correlation between HCV RNA levels in liver, PBMC and serum samples was determined by the non-parametric Spearman Rank Order correlation coefficient. Differences between the two groups of patients were analyzed using the Mann-Whitney U test for non-parametric data. Differences in HCV RNA levels with

respect to demographic characteristics, biochemical and histological indexes of liver damage, HCV genotype (1 vs non-1 genotypes) were analyzed using the Kruskal-Wallis test, while differences with respect to HIV RNA levels (<50 vs >50 copies/ml plasma) and CD4⁺T cell counts (<200 vs ≥200/ml) were analyzed using the Mann-Whitney U test.

All statistical tests were two-sided and *p* values lower than 0.05 were considered statistically significant.

RESULTS

Demographics, biochemical and histologic disease indexes, HCV genotypes and HIV parameters.

Results are reported in Table 1. Median age of patients in group A was lower than in group B ($p < 0.0001$).

Median ALT and AST values as well as the degree of liver inflammation and fibrosis were similar in groups A and B.

In group A, a higher prevalence of genotypes 1 and 4 and a lower prevalence of genotypes 2 and 3 was detected ($p = 0.011$).

HIV infection in patients of group A was well controlled by HAART, as documented by the very low median values of HIV RNA plasma levels and high median CD4⁺ T cell count.

Quantitation of HCV RNA in liver, PBMC and serum.

Results are reported in Table 2.

Upon stratification of each of the 2 groups of patients by demographic characteristics, biochemical indexes of disease activity, HCV genotype, histological activity grade and degree of fibrosis no significant difference of HCV RNA levels was observed between strata in liver, PBMC, or serum.

In group A, neither HIV viral load nor CD4⁺ T cell count did correlate with HCV RNA levels in liver and blood.

In both groups a significant correlation was found between HCV RNA levels in liver and serum (Figure 1). In contrast, HCV RNA levels in PBMC correlated with those in

serum in patients of group A only, and with those in liver in patients of group B only (Figure 2 and 3).

Comparison of liver, PBMC and serum HCV RNA levels in the two groups of patients.

As shown in Figure 4, HCV RNA levels in liver and PBMC were significantly lower in patients of group A than in patients of group B ($p \leq 0.0009$).

HCV RNA serum levels showed an opposite trend being significantly higher in patients of group A than in patients of group B ($p < 0.0001$) (Figure 4).

DISCUSSION AND CONCLUSIONS

As previously reported (9,10), a significant correlation between liver and serum HCV RNA in both groups of patients was found. However, in patients with HIV-HCV coinfection HCV RNA levels in liver were significantly lower, while in serum they were significantly higher than in patients with HCV mono-infection. These findings suggest: i) an enhanced release of HCV in serum, ii) a possible reduction of liver tissue able to support HCV replication. The first hypothesis might be explained by a decreased control of HCV infection in HIV-infected individuals. As for the second hypothesis, liver damage in coinfecting patients might be higher than shown by histology. In this respect, hepatotoxicity of HAART may be an additional factor interfering with liver function and causing further liver damage.

Less consistent was the correlation of HCV RNA levels in PBMC with respect to liver and serum in the two groups of patients. In fact, PBMC HCV RNA levels were shown to correlate with serum HCV RNA levels in patients of group A and with liver HCV RNA levels in patients of group B. Controversial data on the ability of HCV to replicate in PBMC have been reported (13). Our findings do not indicate a substantial contribution of PBMC to total HCV RNA load either in liver or in serum. In addition, no clear difference between the two groups of patients examined was observed. Further investigations are needed to fully elucidate the mechanisms of HCV pathogenesis and clarify

the role of virus replication in different body sites, both in HIV-seronegative and HIV-seropositive individuals.

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Table 1. Characteristics of patients belonging to the two groups examined.

	Group A (n=45) HCV-HIVcoinfection	Group B (n=77) HCV monoinfection
Age (years)		
Median	37	49
Range	31-44	20-66
Sex M/F	37/8	52/25
Biochemical index		
ALT		
Median	78	85
Range	33-270	41-697
AST		
Median	48	47
Range	16-226	24-309
Pathological features		
No hepatitis/ Minimal changes	11 (24.5%)	13 (16.9%)
Mild/ Moderate hepatitis	29 (64.4%)	54 (70.1%)
Severe hepatitis/ Cirrhosis	5 (11.1%)	10 (13.0%)
HAI score		
Median	3	3
Range	0-14	0-12
Fibrosis score		
Median	1	1
Range	0-4	0-4
HCV infection:		
Genotype		
1, 1a, 1b	28 (62.2%)	39 (50.6%)
2a/c, 2b	1 (2.2%)	25 (32.5%)
3a	9 (20.0%)	10 (13.0%)
4, 4c/d	7 (15.6%)	2 (2.6%)
mixture 1+2a/c	-	1 (1.3%)
HIV infection:		
Plasma HIV load (copies/ml)		
Median	<50	NA
Range	<50-369,276	NA
CD4 ⁺ T cells/ μ L blood		
Median	475	NA
Range	166-1,198	NA

HCV, hepatitis C virus; HIV, human immunodeficiency virus; ALT, alanine-aminotransferase, AST, aspartate-aminotransferase; NA, not applicable.

Table 2. HCV RNA levels in liver, PBMC and serum.

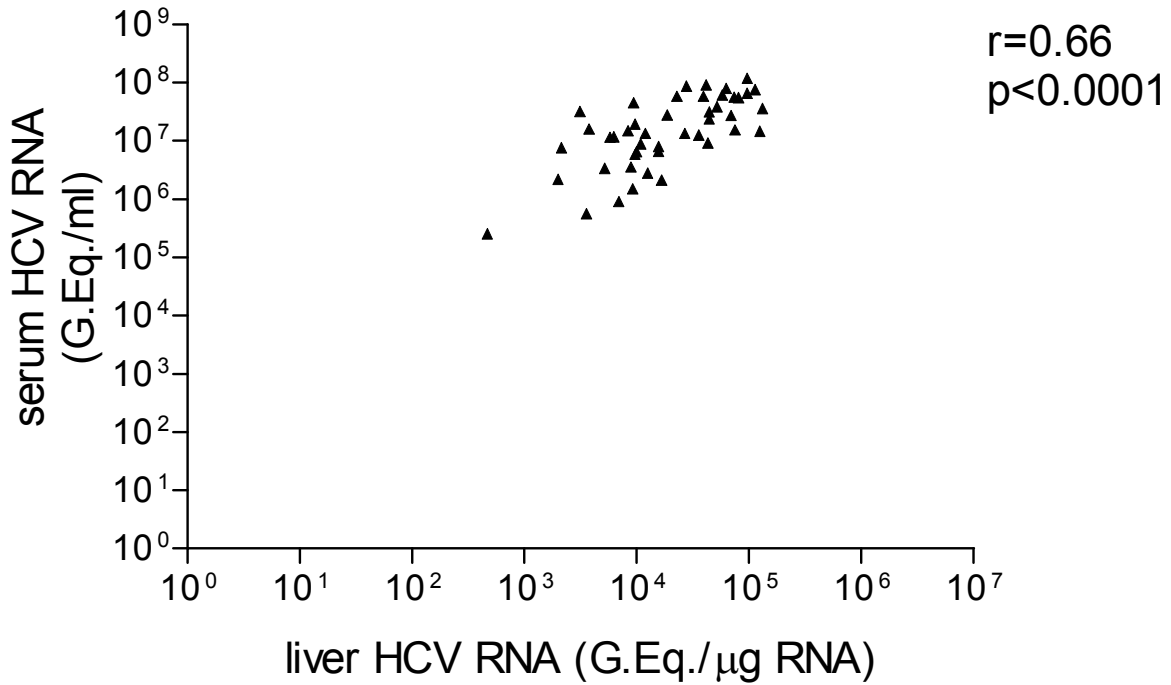
HCV RNA levels	Group A (n=45) HIV-HCV coinfection	Group B (n=77) HCV mono-infection
Intrahepatic ¹		
No. patients	45	77
Median	16,666	78,125
Range	469-131,578	250-1,250,000
PBMC ¹		
No. patients	41	62
Median	937	3,958
Range	0-8,333	0-62,500
Serum ²		
No. patients	45	77
Median	14,940,000	6,104,000
Range	252,000-118,900,000	199,000-74,620,000

HCV, hepatitis C virus; GEq, genome equivalents; PBMC, peripheral blood mononuclear cells;

¹ GEq/ μ gRNA;

² GEq/ml.

Group A (n=45)



Group B (n=45)

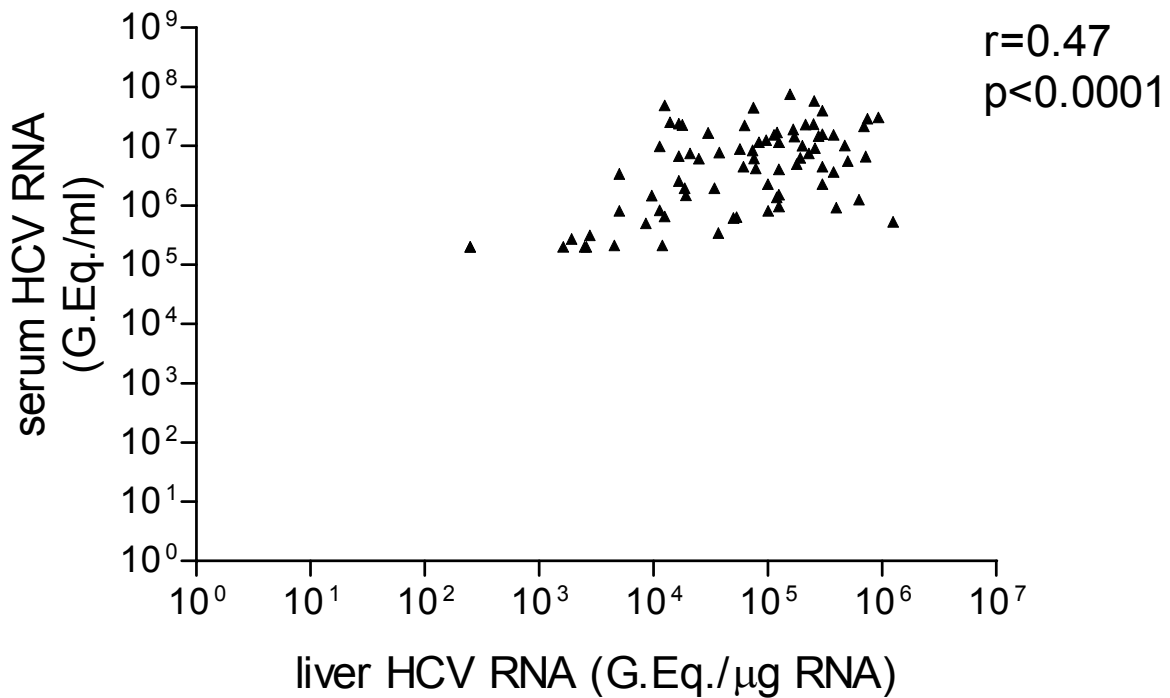
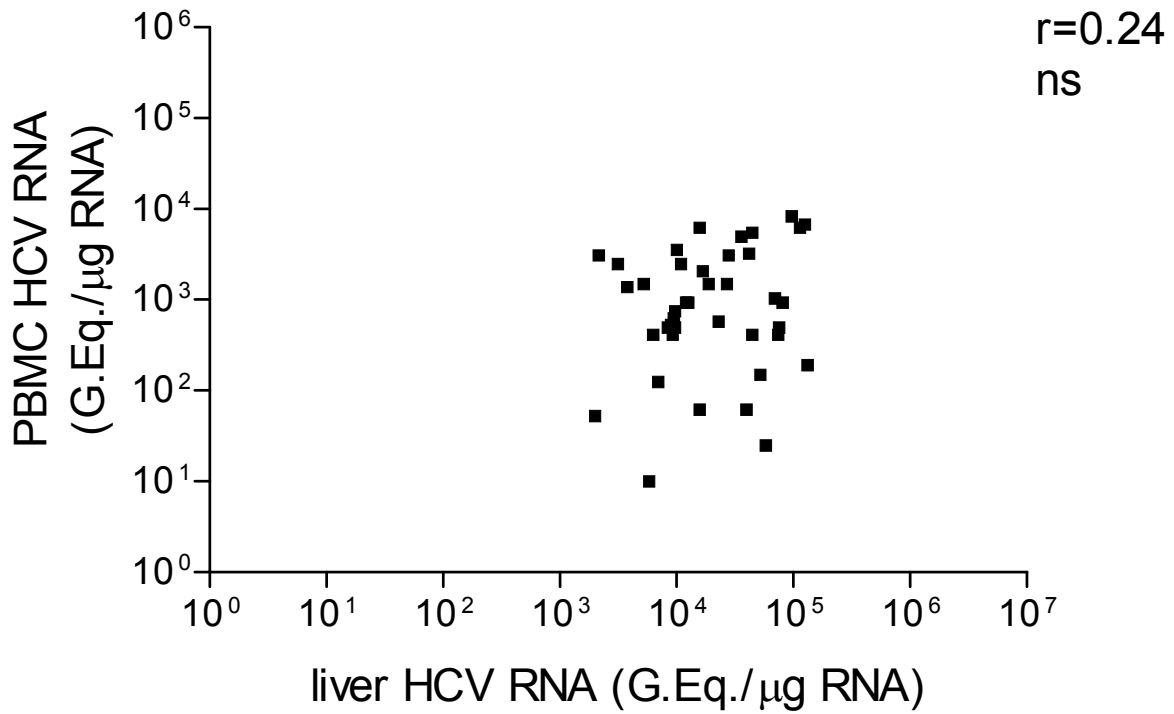


Fig 1. Correlation of HCV RNA levels in liver and serum of HIV-HCV coinfecting (group A) and HCV mono-infected (group B) patients

Group A (n=41)



Group B (n=62)

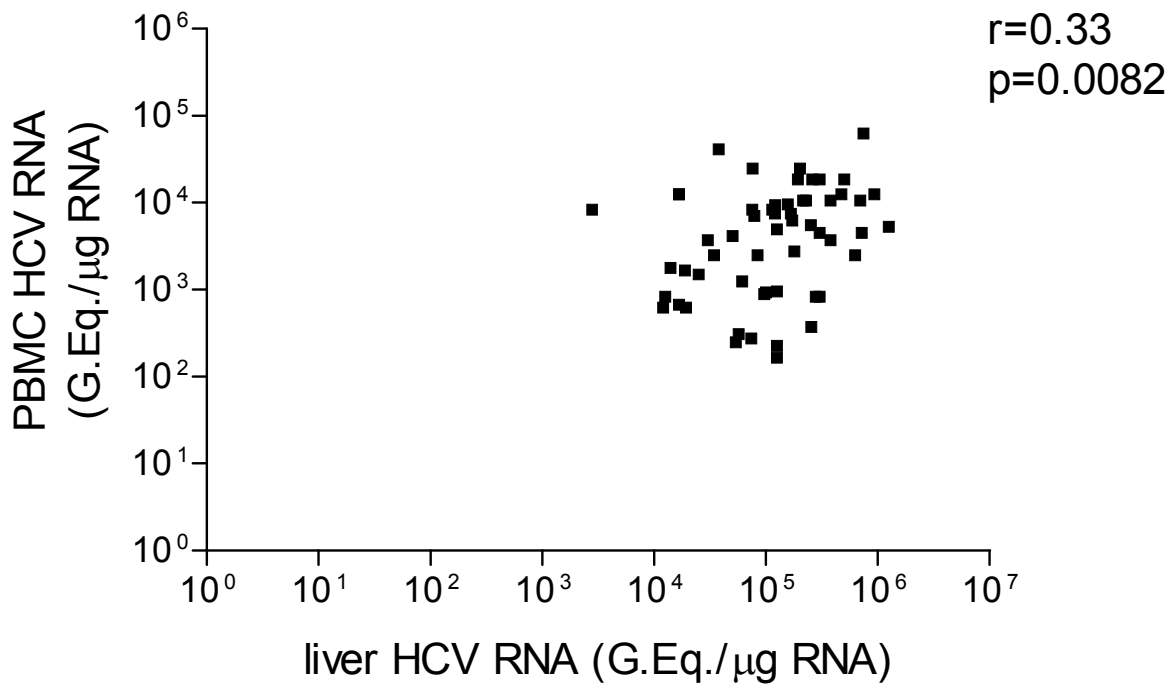
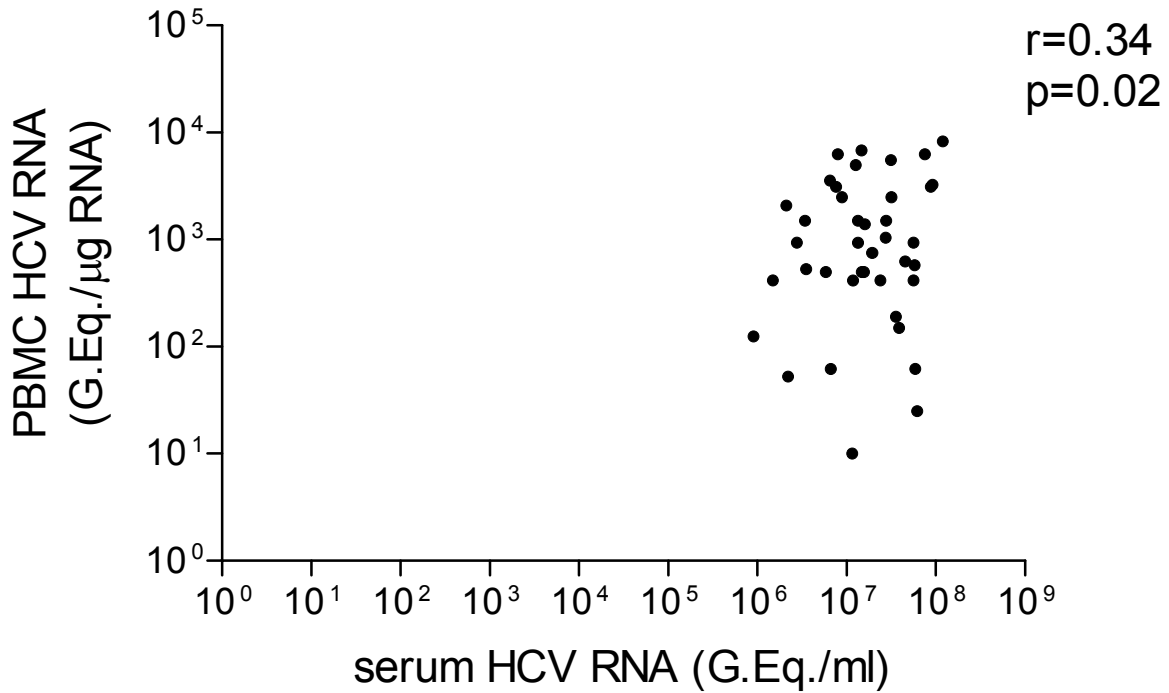


Fig 2. Correlation of HCV RNA levels in liver and PBMC of HIV-HCV coinfecting (group A) and HCV monoinfected (group B) patients.

Group A (n=41)



Group B (n=62)

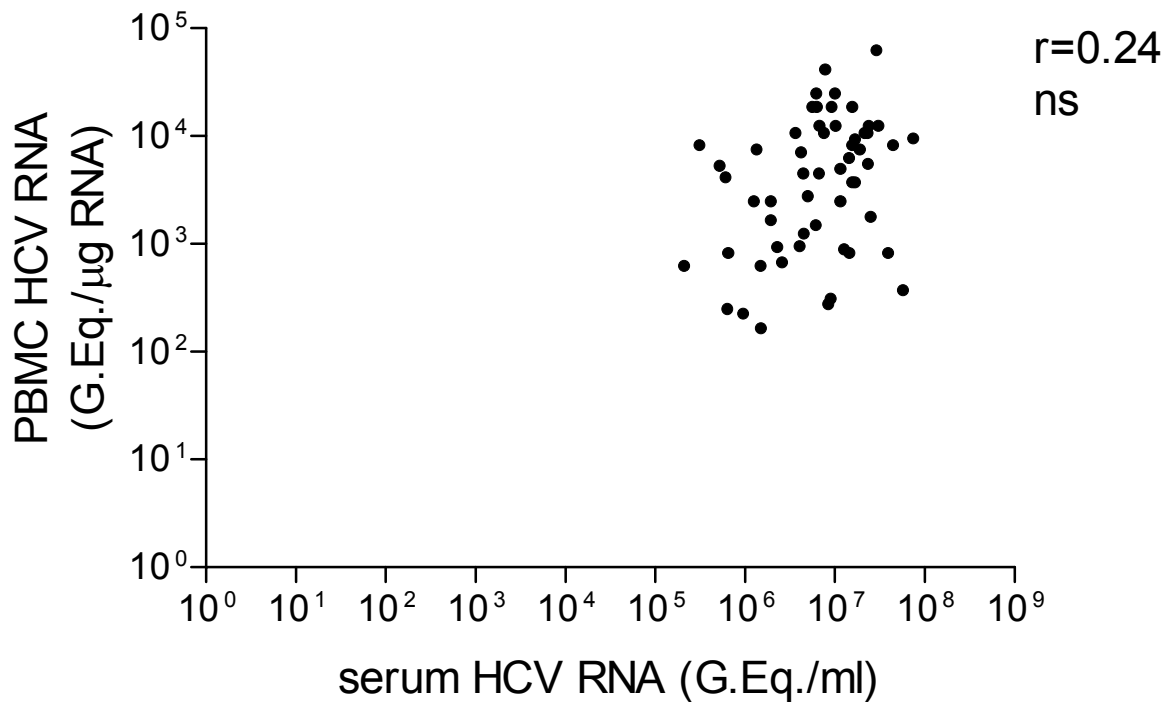


Fig 3. Correlation of HCV RNA levels in serum and PBMC of HIV-HCV coinfecting (group A) and HCV monoinfected (group B) patients.

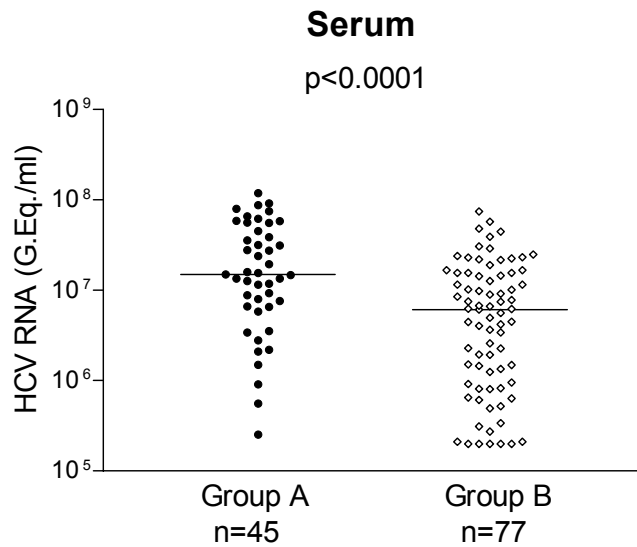
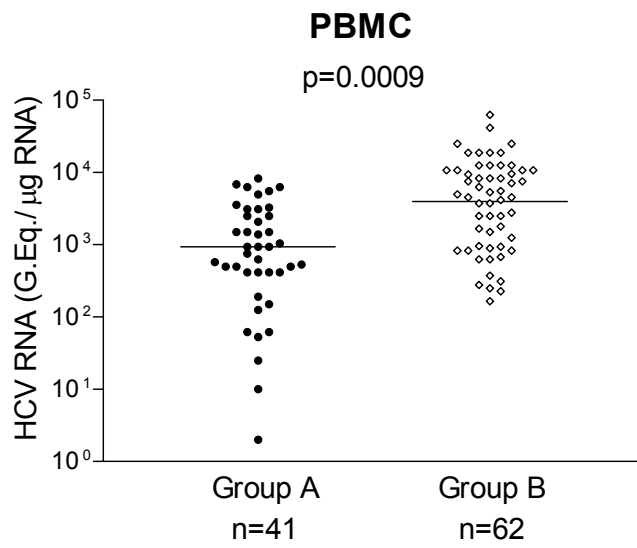
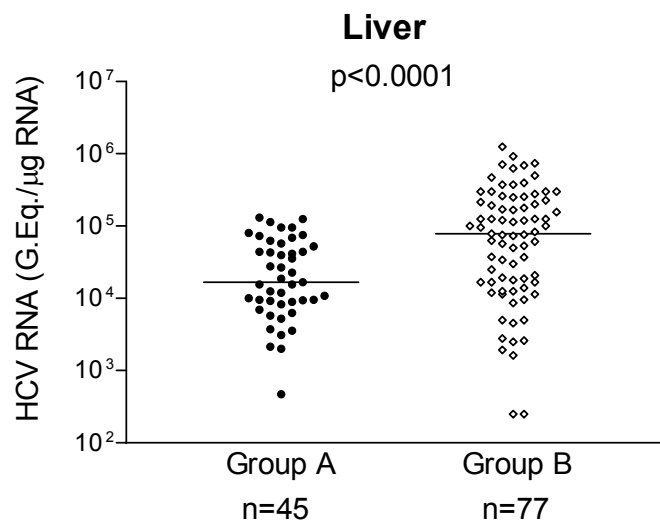


Fig 4. Comparison of liver, PBMC and serum HCV RNA levels of HIV-HCV coinfecting (group A) and HCV mono-infected (group B) patients.