Results: 38 HCV genotype 1b isolates originated in Camporeale have been associated in a unique "transmission cluster". A Monte Carlo Markov simulation, has been used to construct a "bayesian skyline plot" starting from the sequences alignment of the "transmission cluster" isolates. The obtained results have permitted to calculate the date of virus introduction in the population between the end of the 1940s and the begin of the 1950s.
Conclusions: The combination of data obtained from classic phylogenesys and from the application of the Bayes' theorem on the study of the coalescence of genealogical tree are useful to define the spread and possibly the modality of transmission of a pathogen over time. Our data showed that in a small area, such as the Camporeale town, HCV can spread with few circulating strains as a direct consequence of particular events and method of transmission (e.g. glass syringe and transfusion). These data have also shown that the increased medicalization associated to low level of knowledge of the linked risks, as in Italy after world-war II, could have contributed to the diffusion of the hepatitis C infection.

414 ALCOHOL TREATMENT HAS A POSITIVE EFFECT REPLICATION AND PROTEIN EXPRESSION AND THIS EFFECT IS MODULATED BY 5′-UTR REGION

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Background: Alcohol abuse and hepatitis C virus (HCV) infection coexist with chronic liver disease in many patients. Excessive alcohol consumption among patients infected with chronic hepatitis C is likely to result in more severe liver injury, promoting cirrhosis and increasing the risk for development of liver cancer. The mechanisms by which alcohol consumption accelerates liver injury in these patients have not been clearly established.
Aims: Our major aim was to evaluate the effect of alcohol on viral replication and viral protein expression in the HCV replicon system.
Methods: Alcohol effect was evaluated in vitro using Huh-7 hepatoma cells stably expressing the subgenomic HCV RNA flanked by the 5′ and 3′ untranslated regions. We incubated cells with 100–200 mM alcohol for different times of incubation (24, 48 and 72-hour). At the end of this time, viral transcription and protein expression were measured by Semi-quantitative PCR, real-time PCR (by TaqMan) and Western blot (using anti-NPTII and anti-NS5A antisera). Cell viability (tetrazolium salt reduction), AST and ALT levels were also quantified. To evaluate the mechanisms of alcohol-induced HCV expression we used dicistronic constructs bearing the HCV IRES between the protein-coding regions of reporter genes.
Results: HCV replication and expression was positive-induced up to 4.3 times (compared with the control without treatment) using 150 mM alcohol. At this concentration and for these lengths of incubation, alcohol did not appear to induce cytotoxic morphological changes or apoptosis. Interestingly, alcohol also showed a positive-induction of HCV IRES-dependent mRNA translation from dicistronic constructs.
Conclusions: Our results suggest that alcohol treatment has a positive-effect on HCV replication and protein expression, and this effect is modulated by 5-UTR HCV region.

415 EVOLUTION OF HEPATITIS C PLASMA QUASISPECIES AFTER LIVER TRANSPLANTATION DOES NOT REFLECT THAT OF CELL-ASSOCIATED QUASISPECIES IN HUMAN IMMUNODEFICIENCY VIRUS CO-INFECTED PATIENTS

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Compartmentalization of HCV variants is more frequent in liver transplant recipients and in patients infected through drug injection or multiple transfusions. This phenomenon reflects HCV lymphotropism and may have some impact on the course of HCV infection. As liver transplantation, HIV co-infection may favour HCV lymphotropism.
We studied 12 co-infected patients who underwent liver transplantation (LT) for HCV-related cirrhosis. Six serial blood samples were studied: one before LT and 5 within 2 months after LT. Blood cell subsets were separated by immuno-magnetic sorting: CD19+, CD14+, CD8+, CD4/CD25+, CD4/CD25-. Viral loads were assessed by real-time RT-PCR. HCV variants were compared by SSCP. HCV genotype was determined through Line Probe assay.
Plasma HCV RNA was undetectable for 1 patient before LT and for 3 patients during the first week following LT. Thereafter, it was detectable at high levels in all patients. Of the 426 cellular samples, 403 had detectable HCV RNA. Negative samples were mainly observed on day 3 post-LT (10/23), and in pre-transplant samples (6/23). After the third week, all cell samples were positive with viral loads ranging from <1 to 6 log IU/10^6 cells. The subset harbouring the highest cell-associated viral load differed according to time points and patients. In 5 patients, the CD4/CD25+ subset exhibited the highest viral loads though it represents only 10% of the CD4+ population. Cell-associated quasispecies differed from plasma quasispecies in at least one compartment. While no patient had detectable co-infection by different HCV types in plasma, SSCP patterns suggested the presence of an additional strain in the cell-subsets from 6 patients. No significant change was observed for plasma quasispecies in the 12 patients and for cell-associated quasispecies in 5. In 7 patients marked changes were observed in one or more subsets. HIV viral load remained undetectable in all but one patient.
Early after transplantation, stable patterns of plasma quasispecies suggest the absence of selection pressure. Recurrent infection is due to circulating quasispecies. However, cell associated quasispecies may evolve over time. This evolution and the nature of the HCV-infected cells may have a clinical significance that remains to be elucidated.
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416 MOLECULAR ANALYSIS OF GENETIC VARIABILITY AND EPITOPE STABILITY IN A TYPE 1B RECURRENT HCV INFECTION

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A 31 year old man underwent ureteral surgery in august 2002 and developed symptomatic Acute Hepatitis C (ALT 2,460 IU/L, total bilirubin 18 mg/dl, HCV-RNA positive, genotype 1) 45 days after surgery. Seroconversion to anti-HCV defined the etiology in the absence of markers of other etiologies and of other risk factors for acute liver damage. Three plasma samples were stored frozen at –80°C 8, 12 and 18 days after the onset of symptoms. The patient, observed at a three months interval, showed normal ALT and HCV-RNA negative 3, 6 and 9 months after the first observation, but on november 2003 he developed a flare (ALT: 1,740 IU/L, total bilirubin 3.2 mg/dl) and a 4th plasma sample was stored frozen at –80°C. Subsequently the patient developed a biopsy proven chronic C hepatitis.
Molecular analysis of genetic variability and epitope stability was performed in all the 4 plasma samples. Following PCR amplification and molecular sequencing, the partial coding regions corresponding to core, E1, NS3 and NS5 were analysed in MEGA3 Package for nucleotide, synonymous/non-synonymous and amino acid variability. The predicted protein sequences were searched for the presence of experimental or predicted epitopes. The epitope prediction was carried out in SYPHEITY program via web.
A unique isolate was identified from all 4 serial samples. The mean variability of sequences corresponding to the Core, E1 and NS5 regions