

Caring Ambassadors Hepatitis C Program Newsletter
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CLINICAL TRIALS, COHORT STUDIES, PILOT STUDIES

Treatment outcome in relation to alcohol consumption during hepatitis C therapy: An analysis of the Swiss Hepatitis C Cohort Study. Bruggmann P, Dampz M, Gerlach T, et al. Drug Alcohol Depend. 2010 Mar 22. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/20334985>

BACKGROUND: Adherence to hepatitis C treatment is influenced by alcohol as is the action of interferon; yet the clinical significance of the latter remains unclear. The aim of our study was to investigate the influence of ongoing alcohol intake on sustained viral response (SVR) rates in adherent patients receiving hepatitis C treatment. **METHODS:** A retrospective analysis of patients treated with antiviral therapy for hepatitis C infection who were enrolled in the Swiss Hepatitis C Cohort Study was completed. Patients were eligible for the study if they had their HCV RNA tested 6 months following treatment completion and at least one cohort follow-up visit during HCV therapy, documenting the consumed amount of alcohol. They were assigned to three groups according to the amount of alcohol consumption: group A without alcohol consumption, group B ≤ 24 g/d alcohol and group C > 24 g/d alcohol. **RESULTS:** 554 patients were included. Patients with at least 80% of the scheduled cumulative dose and duration did not significantly differ between the three groups. SVR rates according to alcohol consumption were 60% for non-drinkers (group A), 57% in group B and 50% in group C. No significant negative influence from alcohol consumption during therapy was observed in the multiple regression analysis for treatment success. **CONCLUSION:** In this evaluation, we demonstrated comparable SVR rates in non-drinkers and in patients with daily amounts of alcohol intake up to 24g during hepatitis C therapy.

Perihepatic lymph nodes as markers of disease response in patients with hepatitis C-related liver disease: a prospective clinical evaluation. Grier S, Patel N, Kuo YT, et al. Eur J Gastroenterol Hepatol. 2010 Mar;22(3):257-63.

<http://www.ncbi.nlm.nih.gov/pubmed/19177027>

AIM: To assess the clinical feasibility of utilizing the presence of perihepatic lymphadenopathy, seen on ultrasound, as a marker of response to antiviral treatment in patients with hepatitis C virus (HCV)-related liver disease. **METHODS:** Eighty-five patients with HCV-related liver disease [51 men and 34 women; mean age 47 years (range 26-67)] underwent liver biopsy and baseline ultrasound scans. Twenty-two of these patients were followed up longitudinally with 6-monthly ultrasound scans, whereas they were receiving anti-HCV eradication therapy with interferon and ribavirin. Perihepatic lymph nodes detected in the coeliac axis and peripancreatic

region were noted, with the largest node size on maximal diameter recorded. The patients were subsequently assessed in the light of long-term virological response to treatment. **RESULTS:** Perihepatic lymph nodes were detected in 26 of the 85 patients. Of the 22 patients followed up longitudinally, 11 responded to antiviral treatment, nine failed to respond and two did not complete a course of treatment. No significant difference was found between patients with detectable lymphadenopathy and those without according to age, sex, disease severity and genotype. There was a general reduction in size of lymph nodes in both responders and nonresponders to treatment, although this reduction was only significant in the responder group ($P=0.003$). **CONCLUSION:** The presence of perihepatic lymphadenopathy when detected in patients with viral hepatitis can potentially serve as an indicator of response to treatment. However, as only 30-40% of patients have detectable lymphadenopathy, its clinical utility is limited.

Survival in Asian Americans after treatments for hepatocellular carcinoma: a seven-year experience at UCLA. Tong MJ, Chavalitdhamrong D, Lu DS, et al. *J Clin Gastroenterol.* 2010 Mar;44(3):e63-70.

<http://www.ncbi.nlm.nih.gov/pubmed/19745756>

BACKGROUND/RATIONALE: Hepatocellular carcinoma (HCC) is a common malignancy in Asians and is related to the high incidence of chronic viral hepatitis in this ethnic population. The aims of this study were to examine the tumor characteristics and liver disease status in HCC patients of Asian ancestry and determine their survival after treatments for HCC. **RESULTS:** Between September 2000 and December 2007, 278 patients, mean age 61.5 years, presented with HCC to the University of California Los Angeles (UCLA) Liver Cancer Center. Hepatitis B (HBV) infection was detected in up to 68% of Chinese, Korean, and Vietnamese patients, whereas 60% of Japanese patients had Hepatitis C (HCV) infection. Compared with HCC patients who presented with symptoms, those detected by surveillance had more tumors within the Milan and University of California, San Francisco (UCSF) criteria and more patients in Child-Turcotte-Pugh class A. On the basis of a predefined UCLA treatment algorithm, 83% of patients received surgical and/or loco-regional therapies. Compared with other treatments, orthotopic liver transplantation (OLT), and radiofrequency ablation had the highest overall patient survival ($P<0.0001$) and OLT has the highest disease free survival rates ($P<0.0001$). Independent baseline predictors for: (1) patient survival were HBV [hazard ratio (HR) 0.62, $P=0.005$], UCSF criteria (HR 0.46, $P<0.0001$), Child Turcotte Pugh class A (HR 0.57, $P=0.005$), alphafetoprotein per log₁₀ increase (HR 1.26, $P=0.0012$), and alkaline phosphatase per log₁₀ increase (HR 2.32, $P=0.02$); and for (2) disease free survival were UCSF criteria (HR 0.66 $P=0.007$), aspartate aminotransferase per log₁₀ increase (HR 1.50, $P=0.04$), and age per year increase (HR=1.02, $P=0.04$). The 4 Asian subgroups had similar survival rates.

CONCLUSIONS: HBV and Hepatitis C were associated with over 90% of HCC cases in Asian Americans. HCC detected by surveillance identified more patients eligible for surgical and loco-regional therapies, which improved the overall and disease free survival.

Insulin resistance predicts rapid virologic response to peginterferon/ribavirin combination therapy in hepatitis c genotype 4 patients. Khatlab M, Eslam M, Sharwae MA, Shatat M, Ali A, Hamdy L. Am J Gastroenterol. 2010 Mar 16. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/20234345>

OBJECTIVES: In patients with chronic hepatitis C (CHC) of genotype 4, the predictors of rapid virologic response (RVR) have not been determined adequately. We aimed to assess which pretreatment variables might predict an RVR and a sustained virologic response (SVR).

METHODS: A total of 131 non-diabetic, genotype 4 CHC patients were enrolled for analysis and treated with peginterferon-alpha-2b/ribavirin. Insulin resistance (IR) was evaluated by homeostasis model assessment-IR (HOMA-IR). Hepatitis C virus (HCV)-RNA levels were measured at baseline, during therapy and at follow-up. **RESULTS:** The overall SVR rate was 60.3%. The SVR rate in patients with an RVR was 100%. Age, HOMA-IR, fibrosis, severity of the steatosis, and HCV viral load were all significantly associated with RVR in the univariate analysis. After logistic regression, both HOMA-IR (odds ratio: 0.12, P=0.002) and HCV viral load (odds ratio: 1.43, P=0.02) remained independent variables associated with RVR. Age, HOMA-IR, viral load, fibrosis, RVR, and "complete" early virological response were all significantly associated with SVR in the univariate analysis. After logistic regression, fibrosis (odds ratio: 5.23, P=0.007), HOMA-IR (odds ratio: 14.29, P=0.004), and viral load (odds ratio: 0.16, P=0.005) were independent factors associated with SVR. By linear regression, body mass index (P=0.001) and waist circumference (P=0.0003) were independently associated with HOMA-IR. **CONCLUSIONS:** IR is a major determinant of both RVR and SVR in genotype 4 CHC patients. HOMA-IR would seem to be a useful tool for predicting the response to therapy.

Factors that determine the development and progression of gastroesophageal varices in patients with chronic hepatitis C. Fontana RJ, Sanyal AJ, Ghany MG, et al. Gastroenterology. 2010 Mar 5. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/20211180>

BACKGROUND & AIMS: We aimed to identify the incidence and predictors of de novo gastroesophageal variceal formation and progression in a large cohort of patients with chronic hepatitis C (CHC) and advanced fibrosis. **METHODS:** All participants in the HALT-C Trial were offered an endoscopy before treatment and again after 4 years. Patients with varices at baseline also had an endoscopy at 2 years. Baseline laboratory and clinical parameters were analyzed as predictors of de novo variceal formation and variceal progression. **RESULTS:** De novo varices developed in 157 of the 598 (26.2%) patients. Most of the new varices were small (76.4%) and only 1% of patients developed variceal hemorrhage. The likelihood of developing varices was associated with subject race (Hispanic > Caucasian > African American, p= 0.0005), lower baseline levels of albumin (P=0.051), and higher levels of hyaluronic acid (P< 0.001) with an area under the receiver operating characteristic (AUROC) curve=0.70. Among 210 patients with existing gastroesophageal varices, 74 (35.2%) had variceal progression or bleeding during follow-up. Patients with a higher baseline ratios of serum aspartate /alanine aminotransferase (P=0.028) and lower platelet counts (P=0.0002) were at greatest risk of variceal progression (AUROC = 0.72). Prolonged, low-dose peginterferon alpha2a therapy and beta-blockers did not influence the risk of developing new or enlarging varices. **CONCLUSION:** Development of varices in patients with CHC is associated with patient race/ethnicity and laboratory markers of disease severity. Prolonged low dose peginterferon alpha2a therapy and beta-blockers do not reduce the risk of variceal development nor progression.

Re-evaluation of the serum alanine aminotransferase upper normal limit in chronic hepatitis C patients. Yagura M, Tanaka A, Kamitsukasa H, et al. Intern Med. 2010;49(6):525-8. Epub 2010 Mar 15.

<http://www.ncbi.nlm.nih.gov/pubmed/20228585>

OBJECTIVE: The aim of this study was to re-evaluate the upper limit of normal range (ULN) for serum alanine aminotransferase (ALT) in chronic hepatitis C (CH-C) patients who achieved sustained virological response (SVR) to interferon therapy. **METHODS:** Enrolled in this study were 136 consecutive patients, 84 males and 52 females, mean age 52.1+/-14.8 years, with CH-C who received interferon therapy during 1992 to 2008 and achieved SVR. AST and ALT levels (3 serial measurements) were measured every 3 to 4 months over one year after termination of interferon therapy and then the measurements were averaged for each patient. **RESULTS:** The distribution of AST and ALT showed normal distribution. Overall, AST levels were 19.7+/-3 IU/L and ALT levels were 13.8+/-3.1 IU/L in all patients, AST levels were 19.8+/-3 IU/L and 12.9+/-2.9 IU/L and ALT levels were 14.4+/-3.2 IU/L and 9.9+/-3.5 IU/L in male and female patients, respectively. AST level was the highest in the 6th decade and ALT level was in the 5th decade. **CONCLUSION:** In this study on CH-C patients with SVR to interferon therapy, ULN of serum ALT and AST were far lower than the current accepted value. We propose that a suitable ULN of serum AST is <25 IU/L and ALT is <20 IU/L in CH-C patients.

Reduction of liver stiffness by interferon treatment in the patients with chronic hepatitis C. Arima Y, Kawabe N, Hashimoto S, et al. Hepatol Res. 2010 Mar 4. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/20236358>

AIM: To assess the regression of liver fibrosis after interferon (IFN) treatment in patients with chronic hepatitis C, liver stiffness (LS) was measured repeatedly and the factors associated with reduction of LS were assessed. **METHODS:** LS was measured by transient elastography before treatment, at end of treatment (EOT), and 1 year and 2 years after EOT in 145 patients with chronic hepatitis C treated by IFN with or without ribavirin. **RESULTS:** In the patients with sustained virological response (SVR) (n = 93) and relapsers (n = 28), LS significantly decreased at EOT (median, 5.4 [interquartile range, 4.0-8.6] kilopascals [kPa], P < 0.0001 and 6.8 [4.5-8.9] kPa, P = 0.0023) and 1 year after EOT (5.3 [4.2-7.0] kPa, P < 0.0001 and 6.8 [4.5-9.3] kPa, P = 0.0204) compared with baseline (8.0 [5.0-11.9] kPa and 10.6 [7.0-16.6] kPa). In SVR patients, LS significantly decreased 2 years after EOT (5.3 [4.1-6.3] kPa) compared with baseline (P < 0.0001) and LS at EOT (P = 0.0034). Two points or greater reduction of deduced stage at last LS measurement was observed in 78% of SVR patients, 59% of relapsers and 15% of patients with non-virological response whose pretreatment deduced stages were F3-F4. Fibrosis stage, hyaluronic acid levels, duration of treatment, response to treatment and alanine aminotransferase levels were associated with a 2-point or greater decrease of deduced fibrosis stage. **CONCLUSION:** IFN treatment reduced LS in SVR patients and relapsers. Significant reduction of LS is associated with milder fibrosis stage, lower hyaluronic acid levels, longer IFN treatment, virological response of SVR or relapse and higher alanine aminotransferase levels.

Prospective follow-up of patients with acute hepatitis c virus infection in Brazil. Lewis-Ximenez LL, Lauer GM, Schulze Zur Wiesch J, et al. Clin Infect Dis. 2010 Mar 17. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/20235831>

BACKGROUND: The natural outcome of infection with hepatitis C virus (HCV) varies substantially among individuals. However, little is known about host and viral factors associated with a self-limiting or chronic evolution of HCV infection. **METHODS:** From 1 January 2001 through 31 December 2008, a consecutive series of 65 patients from Rio de Janeiro, Brazil, with a well-documented diagnosis of acute HCV infection, acquired via various routes, were enrolled in this study. Patients were prospectively followed up for a median of 40 months after the estimated date of HCV infection with serial measurements of serum alanine aminotransferase, HCV RNA, and anti-HCV antibodies. Spontaneous viral clearance (SVC) was defined as undetectable levels of HCV RNA in serum, in the absence of treatment, for 3 consecutive HCV polymerase chain reaction tests within the first 6 months of follow-up. Cox proportional hazards regression was used to identify host and viral predictors of SVC. **RESULTS:** The cumulative rate of SVC was 44.6% (95% confidence interval, 32.3%-57.5%). Compared with chronic HCV evolution, patients with self-limiting disease had significantly lower peak levels of anti-HCV antibodies (median, 109.0 vs 86.7 optical density-to-cutoff ratio [od/co]; [Formula: see text]), experienced disease symptoms more frequently (69.4% vs 100%; [Formula: see text]), and had lower viral load at first clinical presentation (median, 4.3 vs 0.0 log copies; [Formula: see text]). In multivariate analyses, low peak anti-HCV level (<93.5 od/co) was the only independent predictor for SVC; the hazard ratio compared with high anti-HCV levels (93.5 od/co) was 2.62 (95% confidence interval, 1.11-6.19; [Formula: see text]). **CONCLUSION:** Our data suggest that low levels of anti-HCV antibodies during the acute phase of HCV infection are independently related to spontaneous viral clearance.

Factors associated with uptake of treatment for recent hepatitis C virus infection in a predominantly injecting drug user cohort: The ATACH Study. Grebely J, Petoumenos K, Matthews GV, et al. Drug Alcohol Depend. 2010 Mar 1;107(2-3):244-9.

<http://www.ncbi.nlm.nih.gov/pubmed/19926405>

Despite that the majority of hepatitis C virus (HCV) infection occurs among injection drug users (IDUs), little is known about HCV treatment uptake in this group, particularly during recent infection. We evaluated uptake of treatment for recent HCV infection, including associated factors, within a population predominantly made up of IDUs. The Australian Trial in Acute Hepatitis C was a study of the natural history and treatment of recent HCV infection. All participants with detectable HCV RNA at screening were offered HCV treatment, assessed for eligibility and those initiating treatment were identified. Logistic regression analyses were used to identify predictors of HCV treatment uptake. Between June 2004 and February 2008, 163 were enrolled, with 146 positive for HCV RNA at enrolment. The mean age was 35 years, 77% (n=113) participants had ever injected illicit drugs and 23% (n=34) reported having ever received methadone or buprenorphine treatment. The uptake of HCV treatment was 76% (111 of 146) among those who were eligible on the basis of positive HCV RNA. Estimated duration of HCV infection (OR=1.03 per week, 95% CI=1.00-1.06, P=0.035) and log(10) HCV RNA (OR=1.92 per log(10) increase, 95% CI=1.36-2.73, P<0.001) were independently associated with treatment uptake whereas injection drug use was not. **This study demonstrates** that a high uptake of HCV treatment can be achieved among participants with recently acquired HCV infection. Decisions

about whether to initiate treatment for recently acquired HCV were mainly driven by clinical factors, rather than factors related to sociodemographics or injecting behaviors.

Ribavirin improves early responses to peginterferon through enhanced interferon

signaling. Feld JJ, Lutchman GA, Heller T, et al. Gastroenterology. 2010 Mar 17. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/20303352>

BACKGROUND & AIMS: The therapeutic mechanisms of ribavirin for hepatitis C are unclear. Microarray analyses have shown that ribavirin increases induction of interferon-stimulated genes (ISGs). We evaluated viral kinetics, serum cytokine expression, and viral mutagenesis during early stages of peginterferon therapy with and without ribavirin. **METHODS:** Fifty patients with chronic hepatitis C virus (HCV) infection genotype 1 were randomly assigned to groups that were given peginterferon alfa-2a, with or without ribavirin, for 4 weeks; all patients then received an additional 44 weeks of combination therapy. First- and second-phase viral kinetics were evaluated. Serum levels of IP10, MIG, and MCP1 were quantified as measures of the ISG response. NS5A and NS5B were partially sequenced and mutation rates were calculated.

RESULTS: The first-phase decrease in HCV RNA was similar between groups. Patients that received ribavirin had a more rapid second-phase decrease, compared with patients that did not receive ribavirin-particularly those with an adequate first-phase decrease (0.61 vs. 0.35 log₁₀ IU/mL/week, p=0.018). At 12 hrs, fold induction of serum IP10 was higher in patients given the combination therapy than those given only peginterferon (7.6- vs. 3.8-fold, p=0.01); however, the difference was greatest in patients with an adequate first-phase decrease in HCV RNA. IP10-induction correlated with first- and second-phase kinetics and with ribavirin serum concentrations on day 3. HCV mutation rates were similar between groups. **CONCLUSION:** Ribavirin improves the kinetics of the early response to therapy in patients with an adequate initial response to peginterferon. Induction of interferon-stimulated cytokines correlates with viral kinetics following ribavirin therapy, suggesting that ribavirin promotes interferon signaling.

Increased prevalence of reduced estimated glomerular filtration rate in chronic hepatitis C patients. Petre SA, Sachdev MS, Noble BN, et al. Dig Dis Sci. 2010 Mar 19. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/20300844>

BACKGROUND: To investigate the prevalence and severity of reduced estimated glomerular filtration rate (eGFR) in patients with chronic hepatitis C (CHC). **METHODS:** Medical record review of 831 consecutive CHC patients seen in our clinic between July 2000 and August 2003; eGFR was estimated using the abbreviated Modification of Diet in Renal Disease (aMDRD) equation. The stage of kidney disease was determined based on eGFR expressed in milliliters per minute per 1.73 m²: stage 1 (signs of kidney damage but normal or elevated (eGFR \geq 90), stage 2 (eGFR 60-89), stage 3 (30-59), stage 4 (eGFR 15-29), stage 5 (eGFR < 15 or dialysis-dependent). **RESULTS:** A total of 522 patients had available data with using the aMDRD equation, 51% had abnormal eGFR (stage 1, 4.6%; stage 2, 36.4%; stage 3 or 4, 6.1%; stage 5, 3.8%). Of 190 patients with stage 2 kidney disease, 189 patients (99.5%) had normal serum creatinine and only one patient (0.5%) had elevated creatinine concentrations (>1.4 mg/dl). Of the 32 patients with stage 3 or 4 disease, 20 (62.5%) had a normal serum creatinine concentration. Of 349 patients without diseases known to cause renal insufficiency, 38% had stage 2-4 renal disease. In a subset of these patients, 95/522 (18%) the measured creatinine

clearance showed good correlation with their aMDRD ($R = 0.47$, ($p < 0.0001$)).

CONCLUSIONS: In CHC patients, a normal serum creatinine concentration does not assure normal kidney function. Estimation of eGFR with the aMDRD equation is a more accurate method of identifying patients with chronic kidney disease and reduced eGFR. Therefore, CHC patients should be screened more rigorously for chronic kidney disease because of the high prevalence of reduced eGFR. Lastly, in all CHC patients, the aMDRD eGFR should be used in each encounter with these patients when assessing their renal function irrespective of their serum creatinine.

Pegylated interferon- α , ribavirin, and rituximab combined therapy of hepatitis C virus-related mixed cryoglobulinemia: a long-term study. Dammacco F, Tucci FA, Lauletta G, et al. *Blood*. 2010 Mar 22. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/20308602>

This study illustrates the use and efficacy of a combination of pegylated interferon- α (Peg-IFN- α) and ribavirin (RBV), with or without rituximab (RTX), in hepatitis C virus (HCV)-related mixed cryoglobulinemia (MC). Twenty-two patients with HCV-related MC received Peg-IFN- α (2a: 180 microg or 2b: 1.5 microg/kg) weekly plus RBV (1,000 or 1,200 mg) daily for 48 weeks, and RTX (375 mg/m²) once a week for one month followed by two five-monthly infusions (PIRR for short). Fifteen additional patients received Peg-IFN- α /RBV with the same modalities as the PIRR schedule. Complete response was achieved in 54.5% (12/22) and in 33.3% (5/15) patients who received PIRR and Peg-IFN- α /RBV respectively ($p < 0.05$).

Clearance of HCV RNA and conversion of B cell populations from oligoclonal to polyclonal in the liver, bone marrow and peripheral blood were maintained for up to 3 years in 10/12 (83.3%) and in 2/5 (40%) patients receiving PIRR and Peg-IFN- α /RBV respectively ($p < 0.01$).

Cryoproteins in 22.7% (5/22) patients with PIRR and in 33.3% (5/15) with Peg-IFN- α /RBV persisted despite sustained HCV RNA clearance. No response occurred in the remaining 5 patients of both groups. PIRR therapy is well tolerated and more effective than Peg-IFN- α /RBV combination in HCV-related MC. Its effect may last for over 3 years.

Immunogenicity and safety of different injection routes and schedules of IC41, a Hepatitis C virus (HCV) peptide vaccine. Firbas C, Boehm T, Buerger V, et al. *Vaccine*. 2010 Mar 11;28(12):2397-407. Epub 2010 Jan 9.

<http://www.ncbi.nlm.nih.gov/pubmed/20060945>

BACKGROUND: An effective vaccine would be a significant progress in the management of chronic HCV infections. This study was designed to examine whether different application schedules and injection routes may enhance the immunogenicity of the HCV peptide vaccine IC41. **METHODS:** In this randomized trial 54 healthy subjects received either subcutaneous (s.c.) or intradermal (i.d.) vaccinations weekly (16 injections) or every other week (8 injections). One group additionally received imiquimod, an activator of the toll-like receptor (TLR) 7. The T cell epitope-specific immune response to IC41 was assessed using [(3)H]-thymidine CD4+ T cell proliferation, interferon- γ (IFN- γ) CD8+ and CD4+ ELISpot and HLA-A*0201 fluorescence-activated cell sorting (FACS) tetramer-binding assays. **RESULTS:** More than 60% of vaccinees responded in the CD4+ T cell proliferation assay in all groups. An HLA-A*0201 FACS tetramer-binding assay and IFN- γ CD8+ ELISpot class I response of more than 70% was induced in four and three groups, respectively. IC41 induced significant immunological responses in all groups with responder rates of up to 100%. Interestingly, topical imiquimod was

not able to enhance immunogenicity but was associated with a lower immune response. Local injection site reactions were mostly transient. Intradermal injections caused more pronounced reactions compared to s.c., especially erythema and edema. **CONCLUSION:** Compared to a previous study intensified dosing and/or i.d. injections enhanced the response rates to the vaccine IC41 in three assays measuring T cell function. Immunization with IC41 was generally safe in this study. These results justify testing IC41 in further clinical trials with HCV-infected individuals.

Virus clearance reduces bone fracture in postmenopausal women with osteoporosis and chronic liver disease caused by hepatitis C virus. Arase Y, Suzuki F, Suzuki Y, et al. J Med Virol. 2010 Mar;82(3):390-5.

<http://www.ncbi.nlm.nih.gov/pubmed/20087925>

Osteoporosis is often present in postmenopausal women. The aim of this retrospective cohort study was to assess the cumulative incidence and predictive factors for bone fracture after cessation of interferon (IFN) in postmenopausal women with osteoporosis and chronic liver disease caused by hepatitis C virus (HCV). A total of 420 postmenopausal women treated with IFN monotherapy were enrolled. The mean observation period was 7.2 years. The primary goal was the development of bone fracture. Evaluation was carried out by using the Kaplan-Meier method and the Cox proportional hazards analysis. Thirty-one out of 420 patients sustained bone fracture. The cumulative development rate of bone fracture was 3.6% at 5th year, 9.2% at 10th year, and 17.4% at 15th year. Multivariate Cox proportional hazards analysis showed that bone fracture after cessation of IFN therapy occurred when histological staging of the liver was advanced (hazard ratio (HR): 2.54; 95% confidence interval (CI) = 1.21-5.31; P = 0.013), serum albumin level was < 3.5g/dl (HR: 2.25; 95% CI = 1.10-4.59; P = 0.026), and virus clearance was not achieved (HR: 3.65; 95% CI = 1.11-12.05; P = 0.033). The results indicate that virus clearance causes a reduction of two-thirds in the risk of bone fracture after cessation of IFN therapy in postmenopausal women with osteoporosis and chronic liver disease caused by HCV.

The effect of obesity on intrahepatic cytokine and chemokine expression in chronic hepatitis C infection. Palmer C, Corpuz T, Guirguis M, et al. Gut. 2010 Mar;59(3):397-404. Epub 2009 Mar 15.

<http://www.ncbi.nlm.nih.gov/pubmed/19289381>

BACKGROUND: Obese subjects with chronic hepatitis C virus (HCV) infection have more rapidly progressive liver disease. Objective In this study, we aimed to compare the hepatic cytokine and chemokine profiles in obese and lean subjects with chronic HCV infection using qRT-PCR and immunohistochemistry. **METHODS:** Liver biopsies from 55 subjects were studied, including 20 with chronic hepatitis C, 25 with non-alcoholic fatty liver disease and 10 subjects with non-diseased liver. **RESULTS:** Compared to the control groups, the liver injury in chronic hepatitis C was characterised by increased expression of several T-helper-1 cytokines including interferon-gamma and tumour necrosis factor-alpha, and chemokines such as RANTES, IP-10 and MCP-1. In particular, in comparison with lean (BMI \leq 25 kg/m²) HCV infected subjects, obese (BMI \geq 30 kg/m²) HCV infected subjects had increased hepatic expression of interferon-gamma (p=0.004) and tumour necrosis factor-alpha (p<0.001), as well as increased expression of IP-10 (p=0.009) and MCP-1 (p<0.001). Localisation of these inflammatory chemokines revealed that in comparison to lean-HCV subjects, HCV infected liver from obese subjects exhibited significantly increased expression of IP-10 (p<0.001) and MCP-1

($p=0.02$) in the inflammatory infiltrate of the portal tracts. In parallel, there was increased CD3 infiltration in the liver of obese-HCV subjects. **CONCLUSIONS:** The data provide important mechanistic information on the cause of hepatic injury in obese-HCV subjects including: (1) enhanced T helper-1 cytokine response patterns-to promote hepatocellular injury; (2) increased expression of the chemokines IP-10 and MCP-1 at both the mRNA and protein levels-to enhance inflammatory cell recruitment; (3) differing localisation of these chemokines within the liver of obese-HCV versus lean-HCV subjects-implicating different inducing stimuli and; (4) increased CD3 expression in the liver of obese-HCV subjects-concordant with the increased expression of T cell chemoattractants.

Extending combination therapy with peginterferon plus ribavirin for genotype 2 chronic hepatitis C virological responders: A Pilot Study of 7 Cases. Akuta N, Suzuki F, Arase Y, et al. *Intervirology*. 2010 Mar 3;53(3):188-192. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/20197686>

OBJECTIVE: In treatment-resistant patients with genotype 2 chronic hepatitis C the suitable treatment duration is still unclear. The aims were to investigate extending combination therapy with peginterferon plus ribavirin for genotype 2. **METHODS:** 7 patients infected with genotype 2 at a high viral load and who did not achieve a sustained virological response (SVR) with the first course of 24-week IFN plus ribavirin were recruited into the study protocol with a total of 48 weeks of peginterferon plus ribavirin therapy. **RESULTS:** SVR was achieved in 5 of 7 patients (71%). All 4 patients (100%) who were in relapse with the first course achieved SVR. Only 1 of 3 patients (33%) who had a non-virological response (NVR) with the first course achieved SVR. All 4 patients who had an early virological response (EVR) with the first course achieved EVR and SVR. Two of 3 patients who had no EVR with the first course also did not achieve EVR and SVR. One patient who had no EVR or a NVR during the first course achieved EVR and SVR with the second course. **CONCLUSIONS:** Our results suggest that extending combination therapy for genotype 2 chronic hepatitis C might be useful for patients who relapse following 24-week combination therapy.

Efficacy and safety of pegylated interferon combined with ribavirin for the treatment of older patients with chronic hepatitis C. Huang CF, Yang JF, Dai CY, et al. *J Infect Dis*. 2010 Mar;201(5):751-9.

<http://www.ncbi.nlm.nih.gov/pubmed/20102281>

BACKGROUND: The present study evaluated the efficacy and safety of pegylated interferon (PegIFN)/ribavirin treatment in elderly patients with hepatitis C virus (HCV) infection. **METHODS:** Seventy elderly patients with hepatitis C virus (HCV) infection (group A; age, > 65 years) and 140 sex- and HCV genotype-matched controls (group B; age, 50-64 years) were allocated to receive a PegIFN-alpha-2a/ribavirin standard-of-care regimen. **RESULTS:** Group A had a significantly higher rate of treatment discontinuation (21.4% vs 6.4%; $P = .001$) and grade 3 or 4 adverse events (34.3% vs 20%; $P = .002$) than group B. In intention-to-treat analysis, the sustained virologic response (SVR) rate was substantially lower in group A than in group B (67.1% vs 78.6%; $P = .07$). The inferiority of the SVR rate in group A was observed among patients with HCV genotype 1 (HCV-1) (51.9% vs 75.9%; $P = .03$) but not among patients with HCV genotype 2 or 3 (HCV-2/3) (76.7% vs 80.2%; $P = .65$). Among patients in group A who had a rapid virologic response, those infected with HCV-1 and those infected with HCV-2/3 had similar SVR rates (80% and 87.9%, respectively). For patients receiving treatment for $>80\%$ of

its expected duration, SVR rates were similar between the 2 groups (80.4% vs 82.6%, respectively), regardless of viral genotype. **CONCLUSIONS:** Older patients with HCV infection, especially those in the subgroup infected with HCV-1, had a greater frequency of adverse events and poorer adherence to the standard-of-care regimen, which may be the major reason for treatment inferiority.

Identifying HCV genotype 1 patients at risk of relapse. Deschênes M, Bain VG, Lee SS, Sherman M, et al. Eur J Gastroenterol Hepatol. 2010 Mar 5. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/20216320>

OBJECTIVE: The objective of this analysis was to identify predictors of relapse in genotype 1 patients after 48 weeks of treatment with peginterferon plus ribavirin. **METHODS:** Retrospective analysis of data from treatment-naïve genotype 1 patients with an end-of-treatment response after 48 weeks of treatment with peginterferon alpha-2a plus ribavirin 1000/1200 mg/day in the Canadian Pegasys Expanded Access Program. **RESULTS:** Among treatment-naïve genotype 1 patients with an end-of-treatment response (n = 432), the sustained virological response status was known for 405 individuals (sustained virological response n = 328, 81%; relapse n = 77, 19%). Early virological response rates at week 12 were similar in relapsers (98.7%) and sustained responders (98.5%). More relapsers (12 of 77, 15.6%) than sustained responders (15 of 328, 4.6%) had quantifiable hepatitis C virus (HCV) RNA (≥ 600 IU/ml) at week 12 and, among these patients, mean and maximum HCV RNA levels were higher in relapsers, although the median values were similar. Factors significantly associated with relapse in the multiple logistic regression analysis include older age (odds ratio: 1.48 per decade, 95% confidence interval: 1.06-2.07; P = 0.023), Caucasian ethnicity (odds ratio: 3.23, confidence interval: 1.25-8.33; P = 0.016), higher baseline serum HCV RNA level (P = 0.005), the drop in HCV RNA between baseline and week 12 (P = 0.026), and the interaction between baseline HCV RNA level and the decrease in HCV RNA between baseline and week 12 (P = 0.032). **CONCLUSION:** Older age, Caucasian ethnicity, and high baseline HCV RNA level, and a smaller decrease in HCV RNA between baseline and week 12 predict a relapse in genotype 1 patients.

Thrombocytopenia is more severe in patients with advanced chronic hepatitis C than B with the same grade of liver stiffness and splenomegaly. Tejima K, Masuzaki R, Ikeda H, et al. J Gastroenterol. 2010 Mar 26. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/20339877>

BACKGROUND AND AIM: The mechanism responsible for thrombocytopenia in chronic liver diseases (CLD) is not yet fully understood. The prevalence of thrombocytopenia has been reported to be higher in patients with hepatitis C virus-related hepatocellular carcinoma (CLD-C) than in those with hepatitis B virus-related hepatocellular carcinoma (CLD-B). We have examined the potential difference in thrombocytopenia between patients with CLD-B and those with CLD-C in terms of liver fibrosis adjustment and splenomegaly. **METHODS:** The study cohort consisted of 102 patients with CLD-B and 143 patients with CLD-C were enrolled. Liver stiffness, which is reported to be well correlated with the degree of liver fibrosis, was measured by transient elastography. **RESULTS:** The analysis of covariance with liver stiffness as a covariate revealed that the platelet count was lower in CLD-C patients than in CLD-B patients. Following stratification for liver stiffness, thrombocytopenia was found to be more severe in CLD-C patients than CLD-B patients with advanced liver stiffness, whereas the degree of

splenomegaly was not significantly different. The plasma thrombopoietin level was not different between CLD-B and CLD-C patients with advanced liver stiffness, and the immature platelet number was lower in CLD-C patients despite thrombocytopenia being more severe in these patients. **CONCLUSIONS:** CLD-C patients with advanced liver stiffness presented with more severe levels of thrombocytopenia than CLD-B patients even with the same grade of splenomegaly. Impaired platelet production rather than enhanced platelet destruction may underlie the mechanism responsible for thrombocytopenia in patients with CLD.

Immediate virological response predicts the success of short-term peg-interferon monotherapy for chronic hepatitis C. Yada M, Masumoto A, Yamashita N, et al. *World J Gastroenterol.* 2010 Mar 28;16(12):1506-11.

<http://www.ncbi.nlm.nih.gov/pubmed/20333792>

AIM: To investigate the efficacy of short-term peg-interferon (PEG-IFN) monotherapy for chronic hepatitis C patients who achieved an immediate virological response. **METHODS:** Defining an "immediate virological response (IVR)" as the loss of serum hepatitis C virus (HCV) RNA 7 d after the first administration of PEG-IFN alpha, we conducted a 12-wk course of PEG-IFN alpha2a monotherapy without the addition of ribavirin for 38 patients who had low pretreatment HCV RNA load and exhibited IVR. The patients included 21 men and 17 women, whose ages ranged from 22 to 77 years (mean +/- SD: 52.0 +/- 17.8 years). There were 4 patients with HCV genotype 1b, 23 patients with genotype 2a and 4 patients with genotype 2b. HCV genotype was not determined for the remaining 7 patients. Patients were categorized into a sustained virological response (SVR) group, if serum HCV RNA remained negative for 24 wk after the end of treatment, or into a relapse group. **RESULTS:** Based on the intention-to-treat analysis, 35 patients (92.1%) achieved SVR. One patient (2.6%) relapsed with serum HCV RNA 12 wk after the end of treatment. Two patients (5.3%) withdrew from the study during the 24-wk follow-up period. With regard to the HCV RNA genotype, the SVR rates were 100% (4/4) for genotype 1b, 95.7% (22/23) for genotype 2a and 100% (4/4) for genotype 2b. The SVR rate in 7 patients, whose HCV RNA genotypes were not determined, was 71.4% (5/7). **CONCLUSION:** Short-term PEG-IFN alpha2a monotherapy is highly effective for chronic hepatitis C patients who have low pretreatment HCV RNA load and exhibit IVR.

Hepatitis B virus infection among American patients with chronic hepatitis C virus infection: prevalence, racial/ethnic differences, and viral interactions. Bini EJ, Perumalswami PV. *Hepatology.* 2010 Mar;51(3):759-66.

<http://www.ncbi.nlm.nih.gov/pubmed/20140950>

Little is known about hepatitis B virus (HBV) infection among patients with chronic hepatitis C virus (HCV) infection in the United States. We prospectively enrolled 1,257 patients with chronic HCV infection from two medical centers in New York City. A total of 61.5% (95% confidence interval, 58.8%-64.2%) had evidence of prior exposure to HBV (hepatitis B core antibody-positive), whereas 5.8% (95% confidence interval, 4.5%-7.1%) had dual infection with HBV (hepatitis B surface antigen-positive). Multivariable logistic regression analysis identified age <40 years, Asian race, injection drug use, and a greater number of lifetime sexual partners as independent risk factors for HBV-HCV dual infection. Liver biopsy results in 26 HBV-HCV-infected and 658 HCV-monoinfected patients showed that stage 3 or 4 fibrosis was significantly more common in those with HBV-HCV dual infection (84.6% versus 29.9%; $P < 0.001$). Patients infected with HBV and HCV had significantly lower median HCV RNA levels (1.3

versus 4.5×10^6 copies/mL; $P < 0.001$) and were less likely to have HCV RNA levels $> \text{ or } = 5 \times 10^6$ copies/mL (12.3% versus 45.4%; $P < 0.001$) than those who had HCV mono-infection. All five patients with HBV-HCV dual infection who had undetectable HBV DNA levels had HCV RNA levels $> \text{ or } = 5 \times 10^6$ copies/mL. **CONCLUSION:** American patients with chronic HCV infection should be tested for HBV, especially younger patients, Asians, injection drug users, and those with an increased number of lifetime sexual partners. The presence of severe liver disease and HBV-HCV viral interactions in patients with dual infection necessitates careful but aggressive clinical management, although the optimal strategy remains to be determined.

Mutations within a conserved region of the hepatitis C virus E2 glycoprotein that influence virus-receptor interactions and sensitivity to neutralizing antibodies. Dhillon S, Witteveldt J,

Gatherer D, et al. *J Virol.* 2010 Mar 17. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/20237087>

Cell culture adaptive mutations within the hepatitis C virus (HCV) E2 glycoprotein have been widely reported. We identify here a single mutation (N415D) in E2 that arose during long-term passaging of HCV strain JFH1-infected cells. This mutation was located within E2 residues 412-423, a highly conserved region that is recognized by several broadly neutralizing antibodies, including the mouse monoclonal antibody (MAb) AP33. Introduction of N415D into the wild type (WT) JFH1 genome increased the affinity of E2 to the CD81 receptor and made the virus less sensitive to neutralization by an antiserum to another essential entry factor, SR-BI. Unlike JFH1WT, the JFH1N415D was not neutralized by AP33. In contrast, it was highly sensitive to neutralization by patient-derived antibodies, suggesting an increased availability of other neutralizing epitopes on the virus particle. We included in this analysis viruses carrying four other single mutations located within this conserved E2 region: T416A, N417S and I422L were cell culture adaptive mutations reported previously, while G418D was generated here by growing JFH1WT under MAb AP33 selective pressure. MAb AP33 neutralized JFH1T416A and JFH1I422L more efficiently than the WT virus, while neutralization of JFH1N417S and JFH1G418D was abrogated. The properties of all these viruses in terms of receptor reactivity and neutralization by human antibodies were similar to JFH1N415D, highlighting the importance of the E2 412-423 region in virus entry.

Prevalence and impact of occult hepatitis B infection in chronic hepatitis C patients treated with pegylated interferon and ribavirin. Levast M, Larrat S, Thelu MA, et al. *J Med Virol.*

2010 Mar 24;82(5):747-754. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/20336715>

The prevalence of occult hepatitis B, defined by absence of HBsAg and HBV DNA, ranges widely in patients with hepatitis C. This may influence the treatment of hepatitis C and the severity of liver disease. Sensitive and specific real-time PCR techniques are available commercially and can detect more reliably low HBV DNA levels. The aim of this study was to determine the prevalence of occult hepatitis B virus infection using the COBAS Taqman assay (Roche Diagnostics, Meylan, France) in the serum and liver of HBsAg negative patients with chronic hepatitis C and to evaluate its clinical consequences on liver pathology and its impact on the response to treatment with peg-IFN α and Ribavirin. HBV DNA detection was assessed retrospectively on 140 sera and 113 liver biopsies of HCV positive/HBsAg negative patients before treatment. A 4.4% (5/113) prevalence of occult hepatitis B was recorded in liver samples and in none of the sera. Anti-HBc was not detected in one, three of whom were sustained

virological responders to treatment, one was relapsed responder and one was non-responder. Furthermore, in this cohort composed of 12% anti-HBs negative/anti-HBc positive and 20% anti-HBs positive/anti-HBc positive patients, anti-HBc was not associated with pre-therapeutic viral load, ALT serum levels, and histological activity or fibrosis. Using a commercial real-time PCR assay, we observed a low prevalence of occult B hepatitis. This, just as anti-HBc status, had no clinical impact in a large cohort of hepatitis C patients. It therefore does not appear useful to screen for occult hepatitis B in these patients with this test before beginning HCV treatment. *J. Med. Virol.* 82: 000-000, 2010.

BASIC AND APPLIED SCIENCE, PRE-CLINICAL STUDIES

Gene expression profiling indicates the role of host oxidative stress, apoptosis, lipid metabolism and intracellular transport genes in the replication of hepatitis C virus.

Blackham S, Baillie A, Al-Hababi F, et al. *J Virol.* 2010 Mar 3. [Epub ahead of print]
<http://www.ncbi.nlm.nih.gov/pubmed/20200238>

Hepatitis C Virus is a leading cause of chronic liver disease. The identification and characterisation of key host cellular factors that play a role in the HCV replication cycle is important for the understanding of disease pathogenesis and the identification of novel anti-viral therapeutic targets. Gene expression profiling of JFH-1 infected Huh7 cells by microarray analysis was performed to identify host cellular genes that are transcriptionally regulated by infection. The expression of host genes involved in cellular defence mechanisms (apoptosis, proliferation and anti-oxidant responses), cellular metabolism (lipid and protein metabolism) and intracellular transport (vesicle trafficking and cytoskeleton regulation) was significantly altered by HCV infection. The gene expression patterns identified provide insight into the potential mechanisms that contribute to HCV associated pathogenesis. These include an increase in pro-inflammatory and pro-apoptotic signalling and a decrease in the anti-oxidant response pathways of the infected cell. To investigate whether any of the host genes regulated by infection were required by HCV during replication, siRNA silencing of host gene expression in HCV infected cells was performed. Decreasing the expression of host genes involved in lipid metabolism (TXNIP and CYP1A1) and intracellular transport (Rab33b and ABLIM3) reduced the replication and secretion of HCV indicating that they may be important factors for the virus replication cycle. **These results show** that major changes in the expression of many different genes, in target cells, may be crucial in determining the outcome of HCV infection.

Discovery of vaniprevir (MK-7009), a macrocyclic hepatitis C virus NS3/4a protease

inhibitor. McCauley JA, McIntyre CJ, Rudd MT, et al. *J Med Chem.* 2010 Mar 25;53(6):2443-63.

<http://www.ncbi.nlm.nih.gov/pubmed/20163176>

A new class of HCV NS3/4a protease inhibitors which contain a P2 to P4 macrocyclic constraint was designed using a molecular-modeling derived strategy. Exploration of the P2 heterocyclic region, the P2 to P4 linker, and the P1 side chain of this class of compounds via a modular synthetic strategy allowed for the optimization of enzyme potency, cellular activity, and rat liver exposure following oral dosing. These studies led to the identification of clinical candidate 35b (vaniprevir, MK-7009), which is active against both the genotype 1 and genotype 2 NS3/4a protease enzymes and has good plasma exposure and excellent liver exposure in multiple species.

Hepatitis C virus nonstructural protein 4B: a journey into unexplored territory.

Gouttenoire J, Penin F, Moradpour D. Rev Med Virol. 2010 Mar;20(2):117-29.

<http://www.ncbi.nlm.nih.gov/pubmed/20069613>

Hepatitis C virus (HCV) is a positive-strand RNA virus that replicates its genome in a membrane-associated replication complex. Nonstructural protein 4B (NS4B) induces the specific membrane alteration, designated as membranous web (MW), that harbours this complex. HCV NS4B is an integral membrane protein predicted to comprise four transmembrane segments in its central part. The N-terminal part comprises two amphipathic alpha-helices of which the second has the potential to traverse the membrane bilayer, likely upon oligomerisation. The C-terminal part comprises a predicted highly conserved alpha-helix, a membrane-associated amphipathic alpha-helix and two reported palmitoylation sites. NS4B interacts with other viral nonstructural proteins and has been reported to bind viral RNA. In addition, it was found to harbour an NTPase activity. Finally, NS4B has recently been found to have a role in viral assembly. Much work needs to be done with respect to further dissecting these multiple functions as well as providing a refined membrane topology and complete structure of NS4B. Progress in this direction should yield important insights into the functional architecture of the HCV replication complex and may reveal new opportunities for antiviral intervention against a leading cause of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma worldwide.

Investigating a new generation of ribozymes in order to target HCV. Lévesque MV,

Lévesque D, Brière FP, Perreault JP. PLoS One. 2010 Mar 10;5(3):e9627.

<http://www.ncbi.nlm.nih.gov/pubmed/20224783>

For a long time nucleic acid-based approaches directed towards controlling the propagation of Hepatitis C Virus (HCV) have been considered to possess high potential. Towards this end, ribozymes (i.e. RNA enzymes) that specifically recognize and subsequently catalyze the cleavage of their RNA substrate present an attractive molecular tool. Here, the unique properties of a new generation of ribozymes are taken advantage of in order to develop an efficient and durable ribozyme-based technology with which to target HCV (+) RNA strands. These ribozymes resulted from the coupling of a specific on/off adaptor (SOFA) to the ribozyme domain derived from the Hepatitis Delta Virus (HDV). The former switches cleavage activity "on" solely in the presence of the desired RNA substrate, while the latter was the first catalytic RNA reported to function naturally in human cells, specifically in hepatocytes. In order to maximize the chances for success, a step-by-step approach was used for both the design and the selection of the ribozymes. This approach included the use of both bioinformatics and biochemical methods for the identification of the sites possessing the greatest potential for targeting, and the subsequent in vitro testing of the cleavage activities of the corresponding SOFA-HDV ribozymes. These efforts led to a significant improvement in the ribozymes' designs. The ability of the resulting SOFA-HDV ribozymes to inhibit HCV replication was further examined using a luciferase-based replicon. Although some of the ribozymes exhibited high levels of cleavage activity in vitro, none appears to be a potential long term inhibitor in cellulo. Analysis of recent discoveries in the cellular biology of HCV might explain this failure, as well as provide some ideas on the potential limits of using nucleic acid-based drugs to control the propagation of HCV. Finally, the above conclusions received support from experiments performed using a collection of SOFA-HDV ribozymes directed against HCV (-) strands.

A rapid chemokine response of MIP-1alpha, MIP-1beta and RANTES is associated with a sustained virological response in the treatment of chronic hepatitis C. Florholmen J, Kristiansen MG, Steigen SE, et al. Clin Microbiol Infect. 2010 Mar 6. [Epub ahead of print] <http://www.ncbi.nlm.nih.gov/pubmed/20219081>

The role of chemokines in chronic hepatitis C virus (HCV) infection is not fully understood. The aim of this study was to characterize the baseline serum concentrations and the initial beta-chemokine response to treatment of interferon-alpha and ribavirin with respect to the final clinical outcome of virological response to treatment. Serum concentrations of alanine aminotransferase (ALT) and of the CC subfamily chemokines (macrophage inflammatory protein (MIP)-1alpha, MIP-1beta, monocyte chemoattractant protein (MCP)-1 and Regulated on Activation, Normal T Expressed and Secreted (RANTES)) were measured in patients with chronic HCV infection and in healthy individuals. Necroinflammation and fibrosis were scored in liver biopsies. Treatment outcomes were classified as with or without a sustained virological response after a full course treatment according to the genotypes. The main treatment group consisted of 72 patients with chronic hepatitis C whereas 24 hrs blood samples were available for 41 patients. Increased baseline levels of all CC-chemokines were found in the two responder groups compared to the healthy controls, but reached significant levels only for MIP-1alpha and MCP-1. No correlation was observed between chemokines levels and serum ALT levels, any histological necroinflammatory parameters, or to the fibrosis grade. After 24 hrs of treatment increases of MIP-1alpha, MIP-1beta and RANTES were exclusively observed in the group of sustained virological response. MCP-1 was also significantly increased after 24 hrs in both responder groups, but no differences were observed between the two responder groups. In conclusion, an early response of MIP-1alpha, MIP-1beta, and RANTES may predict a sustained virological treatment response.

Functional characterization of core genes from patients with acute hepatitis C virus infection. Tang X, Wagoner J, Negash A, et al. J Infect Dis. 2010 Mar 15;201(6):912-22. <http://www.ncbi.nlm.nih.gov/pubmed/20170366>

BACKGROUND: The hepatitis C virus (HCV) core protein is implicated in diverse aspects of HCV-induced pathogenesis. There is a paucity of information on core in acute hepatitis C infection. **METHODS:** We analyzed core gene sequences and protein functions from 13 patients acutely infected with HCV genotype 1. **RESULTS:** Although core isolates differed slightly between patients, core quasispecies were relatively homogeneous within each patient. In 2 of 4 patients studied temporally, core quasispecies did not change over time. Comparison with more than 2700 published core isolates indicated that amino acid changes from a prototype reference strain found in acute core isolates were present in chronically infected persons at low frequency (6.4%; range, 0%-32%). Core isolates associated with lipid droplets to similar degrees in Huh7 cells. Core diffusion in cells was not affected by nonconservative changes F130L and G161S in the lipid targeting domain of core. Core isolates inhibited interferon-stimulated response element- and nuclear factor kappaB-dependent transcription and tumor necrosis factor alpha-induced nuclear translocation of nuclear factor kappaB and were also secreted from Huh7 cells. **CONCLUSIONS:** The data suggest that upon transmission, core quasispecies undergo genetic homogenization associated with amino acid changes that are rarely found in chronic infection and that, despite genetic variation, acute core isolates retain similar functions in vitro.

Hepatitis C virus core protein compromises iron-induced activation of antioxidants in mice and HepG2 cells. Moriya K, Miyoshi H, Shinzawa S, et al. *J Med Virol.* 2010 Mar 24;82(5):776-792. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/20336713>

One of the characteristics of hepatitis C virus (HCV) infection is the unusual augmentation of oxidative stress, which is exacerbated by iron accumulation in the liver, as observed frequently in hepatitis C patients. Using a transgenic mouse model, the core protein of HCV was shown previously to induce the overproduction of reactive oxygen species (ROS) in the liver. In the present study, the impact of iron overloading on the oxidant/antioxidant system was examined using this mouse model and cultured cells. Iron overloading caused the induction of ROS as well as antioxidants. However, the augmentation of some antioxidants, including heme oxygenase-1 and NADH dehydrogenase, quinone 1, was compromised by the presence of the core protein. The attenuation of iron-induced augmentation of heme oxygenase-1 was also confirmed in HepG2 cells expressing the core protein. This attenuation was not dependent on the Nrf2 transcription factor. Thus, HCV infection not only induces oxidative stress but also hampers the iron-induced antioxidant activation in the liver, thereby exacerbating oxidative stress that would facilitate hepatocarcinogenesis.

HIV/HCV COINFECTION

Trends in hepatitis C virus infection among patients in the HIV Outpatient Study, 1996-2007. Spradling PR, Richardson JT, Buchacz K, et al. *J Acquir Immune Defic Syndr.* 2010 Mar 1;53(3):388-96.

<http://www.ncbi.nlm.nih.gov/pubmed/19738485>

BACKGROUND: Coinfection with hepatitis C virus (HCV) contributes increasingly to the morbidity and mortality of persons infected with HIV. We assessed HCV infection screening practices and determined trends in the prevalence of HCV infection in the HIV Outpatient Study (HOPS) from 1996 to 2007. **METHODS:** We calculated the proportion of patients eligible to be tested for HCV infection (i.e., never tested or previously tested negative) and the prevalence of HCV infection annually from 1996 to 2007 by sociodemographic, clinical, and HIV risk category characteristics. We used multiple logistic regression analyses to evaluate factors independently associated with HCV testing. **RESULTS:** A total of 7618 patients were active in the HOPS from 1996 through 2007. The proportion of eligible patients tested for HCV infection increased from 10.7% in 1996 to 76.6% in 2007 and increased among all demographic and risk groups. Overall HCV prevalence decreased from 36.7% in 1996 to 19.7% in 2007; decreases in prevalence occurred among all groups except for injection drug users (IDUs). In multivariable analysis, age older than 35 years, nonwhite race, Hispanic ethnicity, high-risk heterosexual and IDU risk categories, and at least 3 years of enrollment in the HOPS were associated with increased odds of having been tested for HCV infection. **CONCLUSIONS:** Screening for HCV infection in the HOPS has improved, although a sizable fraction of patients remain unscreened. The decline in overall HCV infection prevalence from 1996 to 2007 resulted primarily from a decline in the fraction of all prevalent infections in the cohort attributable to IDU patients.

Effect of control selection on sustained viral response rates in genotype 2/3 HCV mono-infected versus HIV/HCV co-infected patients. Nilsson J, Weiland O. *Scand J Infect Dis.* 2010 Mar 10. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/20214543>

Human immunodeficiency virus (HIV) and hepatitis C virus (HCV) co-infected patients have lower rates of sustained viral response (SVR) to treatment than HCV mono-infected patients. A rapid viral response (RVR) with negative HCV-RNA at week 4 predicts SVR in most patients. We evaluated the RVR for the prediction of SVR in mono- and co-infected patients, and the effect caused by the selection of mono-infected controls on SVR rates. Co-infected (n = 13) and mono-infected naïve patients (n = 100) with HCV genotype 2/3 were treated with 135 µg pegylated interferon alpha-2a weekly and weight-based ribavirin daily for 24 weeks. For each co-infected patient, 2 mono-infected controls matched for genotype, baseline viral load, and age, were chosen. RVR was achieved in 6/13 (46%) co-infected, 16/26 (62%) matched controls, and 69/98 (70%) mono-infected patients. All co-infected, 14/16 (88%) matched controls, and 66/69 (96%) mono-infected patients with RVR achieved SVR. In total SVR was reached by 10/13 (77%) co-infected patients and 20/26 (77%) matched controls, somewhat lower than the 86/100 (86%) mono-infected patients (not significant). The ability of RVR to predict SVR was high both in co-infected and mono-infected patients with genotypes 2 and 3 chronic HCV, and the results indicate that co-infected patients with well controlled HIV (with CD4 T-cell counts above 300/µl) can be offered the same treatment as mono-infected patients.

Co-infection of hepatitis B and C viruses and risk of hepatocellular carcinoma: systematic review and meta-analysis. Cho LY, Yang JJ, Ko KP, et al. *Int J Cancer.* 2010 Mar 15. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/20232388>

A sub-additive effect of hepatitis B virus (HBV) and hepatitis C virus (HCV) co-infection is possible since super-infection of one virus tends to inhibit infection of the other virus. However, studies have reported inconsistent findings, and two meta-analyses of studies from various countries (1998) and China (2005) reported a supra-additive effect for hepatocellular carcinoma (HCC) risk. Thus, we re-evaluate HBV/HCV mono-infection and co-infection. Of 411 reports, we included 59 studies that assessed the association between HBV/HCV mono-infection and co-infection for HCC risk. HCC risk due to high/detectable HBV DNA and HBeAg infection was higher than HBsAg infection, while anti-HCV vs. anti-HCV/HCV RNA was not different. Geographically, HCC risk was significantly higher in non-endemic than in HBV or HCV endemic areas. Sub-additive effect for HCC risk was presented in recently published studies, cohort studies, and studies conducted in HBV/HCV non-endemic areas; an additive effect was presented in studies conducted in HBV endemic areas; a supra-additive effect was presented in previously published studies, case-control studies, and studies conducted in HCV endemic areas. Our results suggest HBV/HCV co-infection for HCC risk is not significantly greater than HBV/HCV mono-infection, and HCC risk due to HBV or HCV is higher in non-endemic than endemic areas. P-heterogeneity was significant for most analyses, except HBV(+)/HCV(+) and HBV biomarker analyses. Prevention strategies targeted towards HBV or HCV mono-infected patients are needed. Additionally, tailored prevention to reduce infectivity such as HBV markers (HBeAg, HBV DNA) is needed.

Ability of treatment week 12 viral response to predict long-term outcome in genotype 1 hepatitis C virus/HIV coinfecting patients. Van den Eynde E, Tiraboschi JM, Tural C, et al. AIDS. 2010 Mar 17. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/20299963>

OBJECTIVE: Guidelines recommendation to extend treatment duration in genotype 1 hepatitis C virus (HCV)/HIV-coinfecting patients who clear the virus later than treatment week 4 is not evidence-based. Our main objective was to study the ability of week 12 viral response [early virologic response (EVR)] to predict long-term outcome in patients treated for 48 weeks. **DESIGN::** Multicenter retrospective cohort analysis. **METHODS:** Genotype 1 HCV treatment-naive, HIV-coinfecting adult patients with compensated liver disease who started combination therapy with fixed-dose pegylated-interferon (pegIFN) alfa-2a or weight-based pegIFN alfa-2b plus ribavirin were included. Univariate and forward stepwise logistic regression analysis were used to identify predictors of sustained viral response (SVR) and relapse. **RESULTS:** By intention-to-treat analysis, 31.3% (87/278) of patients achieved an SVR. SVR rate was more than three-fold higher in patients who cleared the virus by week 12 of treatment compared with late responders. Among 123 end-of-treatment responders, 36 (29.3%) relapsed. Relapse risk increased in patients with cirrhosis, in those with ribavirin dose reductions and in late responders: more than 65% of patients who cleared the virus between weeks 12 and 24 relapsed following 48 weeks of treatment compared with 10% of those attaining a complete EVR (<15 IU/ml) at treatment week 12 (risk ratio 6.4, 95% confidence interval 2.9-14.4). **CONCLUSION:** Viral response at treatment week 12 is a strong predictor of long-term outcome. Genotype 1 HCV/HIV-coinfecting patients who achieve a complete EVR (<15 IU/ml) are at low risk of viral relapse after completing the standard 48 weeks of therapy.

Interleukin-27, an Anti-HIV-1 cytokine, inhibits replication of hepatitis C virus. Frank AC, Zhang X, Katsounas A, et al. J Interferon Cytokine Res. 2010 Mar 17. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/20235668>

Interleukin (IL)-27 is a member of IL-12 family cytokine. We have previously reported that IL-27 inhibits human immunodeficiency virus type-1 (HIV-1) replication in CD4(+) T cells and monocyte-derived macrophages, even though IL-12 enhances HIV-1 replication in primary CD4(+) T cells. Further study demonstrates that IL-27 induces antiviral genes including RNA-dependent protein kinase, oligoadenylate synthetase, and myxovirus protein in the same manner as interferon (IFN)-alpha. Neutralization assay using anti-IFN antibodies, real-time RT-PCR, and enzyme-linked immunosorbent assay demonstrated that IL-27 induces the antiviral genes without the induction of IFNs. IFN-alpha has been administered to hepatitis C virus (HCV)-infected patients as well as HCV/HIV-1 co-infected patients. Despite the improved immunotherapy, some patients are still failed to respond to the treatment. Since IL-27 induces IFN-alpha-like responses including the induction of antiviral genes, it was speculated that IL-27 may impact the replication of HCV. In this study, we evaluated the role of IL-27 on HCV replication using Huh7.5, an HCV permissive cell line. IL-27 induces STAT-1 and -3 in the cell line, and dose-dependently inhibited HCV. These data suggest that IL-27 may play a role in the development of a novel immunotherapeutic strategy for HCV and HCV/HIV co-infection.

Hepatitis C virus (HCV) quasispecies complexity and selection in HCV/HIV-coinfected subjects treated with interferon-based regimens. Sherman KE, Rouster SD, Stanford S, et al. *J Infect Dis.* 2010 Mar;201(5):712-9.

<http://www.ncbi.nlm.nih.gov/pubmed/20105080>

BACKGROUND: Coinfection with hepatitis C virus (HCV) and human immunodeficiency virus (HIV) has emerged as a major cause of morbidity and mortality due to liver disease. Interferon-based therapy response rates have been disappointingly low. Baseline HCV complexity and the relationship between complexity and viral kinetic parameters has not been well described in HCV/HIV-coinfected subjects. **METHODS:** A subset of patients enrolled in the AIDS Clinical Trials Group 5071 trial underwent sampling to evaluate viral kinetics and changes in HCV complexity. Early kinetic parameters, baseline complexity, and treatment outcomes--including rapid viral response (RVR), early viral response (EVR), and sustained viral response (SVR)--were evaluated. HCV-monoinfected subjects were matched to HCV/HIV-coinfected subjects. **RESULTS:** Baseline complexity was determined in 108 HCV/HIV-coinfected subjects and in 13 HCV-monoinfected control subjects. Quasispecies complexity was a mean of 2.24 bands for HCV/HIV-coinfected subjects and a mean of 1.90 bands for HCV-monoinfected subjects ($P = .14$). Lower baseline complexity was associated with EVR ($P = .04$) and approached statistical significance for SVR. In patients who underwent viral kinetic modeling, a decrease in complexity was associated with RVR ($P = .03$) and was independent of the correlation between first-phase viral decline efficiency and RVR. **CONCLUSION:** Baseline HCV complexity is an independent predictor of EVR in HCV/HIV-coinfected subjects. A decrease in complexity occurs by 4 weeks after the initiation of interferon-based therapy and is associated with RVR. These findings may enhance the predictive modeling of treatment outcome in HCV/HIV-coinfected patients.

Hepatotoxicity and effectiveness of a Nevirapine-based antiretroviral therapy in HIV-infected patients with or without viral hepatitis B or C infection in Cameroon. Mbougua JB, Laurent C, Kouanfack C, et al. *BMC Public Health.* 2010 Mar 1;10:105.

<http://www.ncbi.nlm.nih.gov/pubmed/20193053>

BACKGROUND: Coinfection with hepatitis B virus (HBV) or hepatitis C virus (HCV) in HIV-infected patients receiving a commonly used nevirapine-based antiretroviral therapy is a major concern for African clinicians owing to its high prevalence, the infrequent testing and treatment of viral hepatitis, and the impact of liver disease on the tolerability and effectiveness of anti-HIV treatment. We compared the hepatotoxicity and the immunological, virological and clinical effectiveness of a nevirapine-based antiretroviral therapy between patients infected with HIV only and patients coinfecting with hepatitis B or C virus in Cameroon. **METHODS:** A retrospective cohort study was conducted among HIV-1-infected patients. Plasma HBV DNA and HCV RNA were tested in positive or indeterminate samples for HBsAg or HCV antibodies, respectively. All patients received nevirapine and lamivudine plus stavudine or zidovudine. **RESULTS:** Of 169 HIV-1-infected patients with a median baseline CD4 count of 135 cells/mm³ (interquartile range [IQR] 67-218), 21% were coinfecting with HBV or HCV. In coinfecting patients, the median viral load was 2.47 x 10⁷ IU/mL for HBV (IQR 3680-1.59 x 10⁸) and 928 000 IU/mL for HCV (IQR 178 400-2.06 x 10⁶). Multivariate analyses showed that the risk of hepatotoxicity was 2-fold higher in coinfecting patients ($p < 0.01$). The response to antiretroviral therapy was however comparable between monoinfected and coinfecting patients in terms of CD4 cell count increase ($p = 0.8$), HIV-1 viral load below 400 copies/mL ($p = 0.9$),

death ($p = 0.3$) and death or new AIDS-defining event ($p = 0.1$). Nevirapine was replaced by a protease inhibitor in 4 patients owing to hepatotoxicity. **CONCLUSION:** This study suggests that the nevirapine-based antiretroviral therapy could be used safely as first-line treatment in patients with low CD4 cell count in Africa despite frequent coinfections with HBV or HCV and infrequent testing of these infections. Although testing for HBV and HCV should be systematically performed before initiating antiretroviral therapy, transaminases elevations at baseline or during treatment should be a decisive argument for testing when hepatitis status is unknown.

Rate and predictors of success in the retreatment of chronic hepatitis C virus in HIV/hepatitis C Virus coinfecting patients with prior nonresponse or relapse. Labarga P, Vispo E, Barreiro P, et al. *J Acquir Immune Defic Syndr.* 2010 Mar 1;53(3):364-8.
<http://www.ncbi.nlm.nih.gov/pubmed/20101191>

BACKGROUND: In hepatitis C virus (HCV)/HIV-coinfecting patients who failed a course of suboptimal hepatitis C therapy, retreatment with adequate doses and duration of pegylated interferon (pegIFN) plus ribavirin (RBV) is advisable in the presence of compensated advanced liver fibrosis. **METHODS:** The efficacy and safety of pegIFN-alpha2a (180 microg/wk) plus RBV (<75 kg: 1000 mg/d; > or = 75 kg: 1200 mg/d) given for 12 months was prospectively assessed in HIV/HCV patients with nonresponse or relapse to a prior course of suboptimal hepatitis C therapy. The main endpoint was the achievement of sustained virological response (SVR). **RESULTS:** A total of 52 patients were enrolled in the study (78% HCV genotypes 1 or 4; 56% with advanced liver fibrosis). Prior suboptimal regimens were IFN monotherapy (20%), IFN plus RBV (29%), and pegIFN plus RBV 800 mg/d (51%). Overall, 61% were nonresponders and 39% relapsers. Retreatments provided SVR in 30.8% of patients (19.5% for genotypes 1/4 vs. 72.7% for genotypes 2/3; $P = 0.002$). In multivariate analysis, HCV genotypes 2/3 [OR 22.2, 95% confidence interval (CI), 2.9-166.7, $P = 0.003$] and RBV plasma trough concentrations at week 4 [OR 3.9 (95% CI, 1.3-11.8), $P = 0.01$] were the only independent predictors of SVR. **CONCLUSIONS:** Retreatments with pegIFN-alpha2a plus weight-based RBV for 12 months permits to achieve HCV clearance in nearly one third of HIV/HCV-coinfecting patients who failed a prior suboptimal course of hepatitis C therapy. Patients with HCV genotypes 2/3 and those with RBV plasma trough levels above 2.07 microg/mL show the highest chances of SVR.

HIV/Hepatitis C virus-coinfecting virologic responders to pegylated interferon and ribavirin therapy more frequently incur interferon-related adverse events than nonresponders do. Osinusi A, Rasimas JJ, Bishop R, et al. *J Acquir Immune Defic Syndr.* 2010 Mar 1;53(3):357-63.

<http://www.ncbi.nlm.nih.gov/pubmed/20101190>

BACKGROUND: This study aimed to assess the relationship between interferon (IFN)-related adverse effects and Hepatitis C virus (HCV) virologic response in HIV/HCV-coinfecting individuals treated with pegylated interferon and ribavirin. **METHODS:** We conducted 2 prospective, open-label trials treating HIV/HCV-coinfecting individuals with pegylated interferon alpha-2b or alpha-2a and ribavirin for 48 weeks. Safety laboratories, HCV RNA, psychiatric, and ophthalmologic evaluations were performed at baseline and monthly until week 72. **RESULTS:** Responders were defined as those with HCV RNA decline of > or = 2-log drop from baseline and nonresponders were those who did not. Remarkably, of the 27 patients (50%) who developed psychiatric toxicities, 26 patients were responders, although only 1 of 14 virologic nonresponders

experienced psychiatric toxicity. Other adverse effects, such as anemia and ophthalmologic toxicities, were also more frequent in responders compared with nonresponders. Decline in CD4 T-cell counts strongly correlated with HCV viral decline. **CONCLUSIONS:** Our study demonstrates coupling of antiviral effect and occurrence of adverse events in HIV/HCV-coinfected patients. These patients with IFN-related adverse effects need a multidisciplinary treatment approach, hence, they are more likely to achieve sustained virologic response. Future studies are needed to evaluate the factors that predict the development of IFN-alpha-dependent adverse events before therapy.

Survival of HIV-infected patients with compensated liver cirrhosis. Tuma P, Jarrin I, Del Amo J, et al. AIDS. 2010 Mar 13;24(5):745-53.

<http://www.ncbi.nlm.nih.gov/pubmed/20154579>

INTRODUCTION: Since the advent of HAART, liver-related mortality has become the leading cause of non-AIDS deaths in HIV-infected patients in western countries, complications of end-stage liver disease due to chronic hepatitis B, chronic hepatitis C or both being mainly responsible. **METHOD:** The incidence and predictors of mortality were examined in HIV-infected patients with compensated liver cirrhosis. The accuracy of three different methods (elastometry, Child-Pugh and Model for End-Stage Liver Disease scores) to predict mortality was further examined. Cirrhosis was defined for hepatic elastometry values above 14.5 kPa. **RESULTS:** A total of 194 (11.4%) out of 1706 HIV-positive individuals were cirrhotic and were prospectively followed since October 2004 until December 2008. Overall, 89% of cirrhotic individuals had chronic hepatitis C, 10.3% chronic hepatitis B, 4.6% hepatitis delta and 4.1% liver disease of other causes or unknown cause. The overall mortality rate was 5.8 deaths per 100 patient-years. Multivariate analyses showed that age of at least 50 years (hazard ratio 4.76, 95% confidence interval 1.66-13.59, $P = 0.004$), CD4 cell counts below 200 cells/microl (hazard ratio 3.01, 95% confidence interval 1.26-7.23, $P = 0.03$) and detectable plasma HIV-RNA (hazard ratio 3.97, 95% CI, 1.53-10.27, $P = 0.005$) were associated with mortality. A baseline Model for End-stage Liver Disease score of at least 11 ($P = 0.03$) and hepatic elastometry values above 28.75 kPa ($P = 0.001$) were independent predictors of mortality. **CONCLUSION:** The death rate in HIV-infected patients with compensated liver cirrhosis in the HAART era is 5.8% yearly, higher than mortality previously reported for either HIV-uninfected individuals with cirrhosis or noncirrhotic HIV-positive patients. Factors associated with mortality were older age, low CD4 cell counts and detectable plasma HIV-RNA. Both Model for End-Stage Liver Disease and especially hepatic elastometry accurately predicted mortality in this population.

Activation of CD8 T cells predicts progression of HIV infection in women coinfecting with hepatitis C virus. Kovacs A, Karim R, Mack WJ, et al. J Infect Dis. 2010 Mar 15;201(6):823-34.

<http://www.ncbi.nlm.nih.gov/pubmed/20151840>

BACKGROUND: Because activation of T cells is associated with human immunodeficiency virus (HIV) pathogenesis, CD4 and CD8 activation levels in patients coinfecting with HIV and hepatitis C virus (HCV) may explain conflicting reports regarding effects of HCV on HIV disease progression. **METHODS:** Kaplan-Meier and multivariate Cox regression models were used to study the risk of incident clinical AIDS and AIDS-related deaths among 813 HCV-negative women with HIV infection, 87 HCV-positive nonviremic women with HIV coinfection, and 407 HCV-positive viremic women with HIV coinfection (median follow-up time, 5.2 years).

For 592 women, the percentages of activated CD4 and CD8 T cells expressing HLA-DR (DR) and/or CD38 were evaluated. **RESULTS:** HCV-positive viremic women had a statistically significantly higher percentage of activated CD8 T cells ([Formula: see text]) and a statistically significantly higher incidence of AIDS compared with HCV-negative women ([Formula: see text] [log-rank test]). The AIDS risk was greater among HCV-positive viremic women in the highest tertile compared with the lowest tertile (>43% vs <26%) of CD8(+)/CD38(+)/DR(+) T cells (hazard ratio, 2.94 [95% confidence interval, 1.50-5.77]; [Formula: see text]). This difference was not observed in the HCV-negative women (hazard ratio, 1.87 [95% confidence interval, 0.80-4.35]; [Formula: see text]). In contrast, CD4 activation predicted AIDS in both groups similarly. Increased percentages of CD8(+)/CD38(-)/DR(+), CD4(+)/CD38(-)/DR(-), and CD8(+)/CD38(-)/DR(-) T cells were associated with a >60% decreased risk of AIDS for HCV-positive viremic women and HCV-negative women. **CONCLUSION:** HCV-positive viremic women with HIV coinfection who have high levels of T cell activation may have increased risk of AIDS. Earlier treatment of HIV and HCV infection may be beneficial.

Hepatic steatosis associated with increased central body fat by dual-energy X-ray absorptiometry and uncontrolled HIV in HIV/hepatitis C co-infected persons. Brown TT, Mehta SH, Sutcliffe C, et al. AIDS. 2010 Mar 27;24(6):811-7.

<http://www.ncbi.nlm.nih.gov/pubmed/20186036>

OBJECTIVE: To evaluate the relationship between regional body composition and liver disease (fibrosis or steatosis) in HIV/HCV co-infected individuals. **METHODS:** Whole body dual-energy X-ray absorptiometry (DXA) was performed in 173 HIV/HCV co-infected persons within 12 months of a liver biopsy. Significant fibrosis was defined as a METAVIR stage greater than 1. Steatosis was graded as: 0, none; 1, steatosis involving less than 5% of hepatocytes; 2, 5-29%; 3, 30-60%; 4 greater than 60%, and was defined as more than 0. Poisson regression with robust variance was used to estimate prevalence ratios of the outcome measures. **RESULTS:** The population was 62% male and 84% black with a median body mass index of 25.2 kg/m (interquartile range 22.5, 29.3 kg/m). No differences in regional body fat or fat distribution were observed in 42 patients with significant fibrosis compared to others with less fibrosis. However, the 77 individuals (45%) with steatosis had greater central fat than those without steatosis [prevalence ratio 1.04 per kg trunk fat; 95% confidence interval (CI) 1.04, 1.11], after adjusting for hepatic fibrosis (prevalence ratio 1.77; 95% CI 1.29, 2.42), uncontrolled HIV replication (viral load >400 copies/ml) (prevalence ratio 1.57; 95% CI 1.12, 2.22), age, sex, race and diabetes mellitus. **CONCLUSIONS:** In HIV/HCV co-infected individuals, measures of regional body fat or fat distribution were not associated with hepatic fibrosis. In contrast, increased central adiposity by DXA, as well as concomitant fibrosis and uncontrolled HIV, were associated with hepatic steatosis. The extent to which weight loss and effective antiretroviral therapy can reduce the risk of steatosis deserves further investigation.

Impact of highly active antiretroviral therapy on hepatitis C virus protease quasispecies diversity in HIV co-infected patients. Winters MA, Chary A, Eison R, et al. J Med Virol. 2010 Mar 24;82(5):791-798. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/20336744>

Many hepatitis C virus (HCV)-infected patients are also infected with HIV, and undergo antiretroviral (ARV) treatment for their human immunodeficiency virus (HIV) infection. Due to changes in HIV burden and immunologic status, HIV ARV treatment may have indirect effects

on the HCV population, which could impact the effectiveness of subsequent HCV protease inhibitor (PI) treatment. The genetic variability of the protease-encoding HCV NS3 gene was evaluated in 10 co-infected patients initiating ARVs (both before and after ARV initiation), and compared to the genetic variability in 10 patients on stable ARV therapy. After RT-PCR of plasma-derived HCV RNA, a mean of 20 clones per patient time-point were sequenced and analyzed for changes in the HCV quasispecies population. No significant differences in sequence diversity or complexity at the nucleic acid or amino acid levels were seen at baseline between groups or between the two time points in either group. HCV protease diversity in the pre- and post-ARV treatment samples was not significantly different than samples from patients on stable ARV therapy. There was no significant development of amino acid substitutions known to confer HCV PI resistance in either group. Initiation of ARV for HIV infection does not significantly alter the genetic diversity or complexity of the HCV NS3 gene or result in increased number of HCV PI-associated amino acid changes. These results suggest ARV treatment for HIV would not affect the efficacy of HCV PI treatment.

COMPLEMENTARY AND ALTERNATIVE MEDICINE

Identification of hepatoprotective flavonolignans from silymarin. Polyak SJ, Morishima C, Lohmann V, et al. Proc Natl Acad Sci U S A. 2010 Mar 15. [Epub ahead of print]
Pal S, Lee DY, Liu Y, Graf TN, Oberlies NH.

<http://www.ncbi.nlm.nih.gov/pubmed/20231449>

Silymarin, also known as milk thistle extract, inhibits hepatitis C virus (HCV) infection and also displays antioxidant, anti-inflammatory, and immunomodulatory actions that contribute to its hepatoprotective effects. In the current study, we evaluated the hepatoprotective actions of the seven major flavonolignans and one flavonoid that comprise silymarin. Activities tested included inhibition of: HCV cell culture infection, NS5B polymerase activity, TNF-alpha-induced NF-kappaB transcription, virus-induced oxidative stress, and T-cell proliferation. All compounds were well tolerated by Huh7 human hepatoma cells up to 80 muM, except for isosilybin B, which was toxic to cells above 10 muM. Select compounds had stronger hepatoprotective functions than silymarin in all assays tested except in T cell proliferation. Pure compounds inhibited JFH-1 NS5B polymerase but only at concentrations above 300 muM. Silymarin suppressed TNF-alpha activation of NF-kappaB dependent transcription, which involved partial inhibition of IkappaB and RelA/p65 serine phosphorylation, and p50 and p65 nuclear translocation, without affecting binding of p50 and p65 to DNA. All compounds blocked JFH-1 virus-induced oxidative stress, including compounds that lacked antiviral activity. The most potent compounds across multiple assays were taxifolin, isosilybin A, silybin A, silybin B, and silibinin, a mixture of silybin A and silybin B. The data suggest that silymarin- and silymarin-derived compounds may influence HCV disease course in some patients. Studies where standardized silymarin is dosed to identify specific clinical endpoints are urgently needed.

Institute of Medicine recommendations for the prevention and control of hepatitis B and C.

Mitchell AE, Colvin HM, Palmer Beasley R. Hepatology. 2010 Mar;51(3):729-33.

<http://www.ncbi.nlm.nih.gov/pubmed/20186842>

Despite federal, state, and local public health efforts to prevent and control hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, these diseases remain serious health problems in the United States. About 1%-2% of the U.S. population has chronic HBV or HCV infections, and each year about 15,000 people die from liver cancer or liver disease related to these preventable infections. The Institute of Medicine formed an expert committee to determine ways to reduce new HBV and HCV infections and the morbidity and mortality related to chronic viral hepatitis and released its findings in a report. The major factor found to impede current efforts to prevent and control HBV and HCV is lack of knowledge and awareness about these diseases among healthcare and social-service providers, members of the public, and policy makers. Because the extent and seriousness of this public health problem is not appreciated, inadequate resources are being allocated to prevention, control, and surveillance programs. This situation has led to continued transmission of HBV and HCV and inadequate identification of and medical management for chronically infected people. **CONCLUSION:** To address the situation, the Institute of Medicine report makes recommendations in four areas: improved surveillance for HBV and HCV; improved knowledge and awareness among healthcare and social-service providers and the public, especially at-risk people; improved HBV vaccine coverage; and improved viral hepatitis services and access to those services.

Dried blood spot for hepatitis C virus serology and molecular testing.

Tuaillon E, Mondain AM, Meroueh F, et al. Hepatology. 2010 Mar;51(3):752-8.

<http://www.ncbi.nlm.nih.gov/pubmed/20043287>

We investigated the performance of dried blood spots (DBS) in hepatitis C virus (HCV) diagnosis using modified commercial tests. Paired DBS and serum samples were collected from 200 patients: 100 patients with anti-HCV antibodies (anti-HCV), including 62 patients with detectable serum HCV RNA, and 100 patients without anti-HCV. The DBS sample consisted of three drops of approximately 50 microL of whole blood applied to a paper card, which was then stored at -20 degrees C within 48 hours of collection. Using the Ortho HCV 3.0 enzyme-linked immunosorbent assay kit on DBS, we observed both a specificity and sensitivity of 99% in detecting anti-HCV. HCV RNA was detected on DBS in 60/62 (97%) patients with detectable serum HCV RNA, which was then successfully quantified in 55 samples (89%) using the Cobas TaqMan HCV test. A good correlation was observed between the DBS HCV RNA concentration and the serum level ($r(2) = 0.95$; $P < 0.001$). HCV genotyping was successfully performed on DBS samples, with a full concordance between the 14 paired DBS and serum samples (genotypes 1-4). **CONCLUSION:** This study presents DBS as a reliable alternative to serum specimens for detecting anti-HCV, quantifying HCV RNA and genotyping HCV. DBS may increase the opportunities for HCV testing and treatment follow-up in hard-to-reach individuals.

Methadone maintenance patients' knowledge, attitudes, beliefs, and experiences concerning treatment for hepatitis C virus infection. Canfield KM, Smyth E, Batki SL. *Subst Use Misuse*. 2010 Mar;45(4):496-514.

<http://www.ncbi.nlm.nih.gov/pubmed/20141461>

Hepatitis C virus (HCV) knowledge, attitudes, beliefs, and experiences (KABE) of 64 HCV antibody positive methadone maintenance treatment (MMT) patients were assessed in conjunction with acceptability of an on-site semi-structured HCV education session, HCV RNA diagnostic testing, HCV treatment motivational assessment, and initiation of HCV treatment. The KABE interviews were conducted in 2006 and 2007 in an urban New York State MMT clinic in affiliation with a NIDA-funded HCV research project. The majority had basic knowledge of HCV disease, but poor understanding of HCV testing and treatment. While the majority of participants expressed fear of HCV treatment side effects, 88% accepted HCV RNA testing and 78% expressed willingness to start HCV treatment with the majority of chronically infected choosing to start HCV treatment medications. Study limitations and implications are discussed.

Uncertainty and patients' preferred role in decision making. Fraenkel L. *Patient Educ Couns*. 2010 Mar 20. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/20307951>

OBJECTIVE: Experts recommend that clinicians tailor their interactions according to each patient's preferred decision style. Because participation is associated with improved clinical outcomes, factors which modify preferred role should be addressed before determining the level at which patients wish to participate. The objective of this study was to determine if certainty related to initiating treatment is related to preferred role in decision making. **METHODS:** We conducted face-to-face interviews with 142 patients eligible for the treatment of hepatitis C. Preferred role in decision making was measured using the Control Preferences Scale and certainty was measured with 11-point numeric rating scale. **RESULTS:** Subjects who were uncertain whether they wanted to start treatment were more likely to prefer Role 2: "to make the final selection of my treatment after seriously considering my doctor's opinion" over Role 1 "to make the final selection about which treatment I will receive" compared to those who were certain [Adjusted OR (95% CI)=4.9 (1.7-14.5)]. Subjects who were uncertain were also more likely to prefer sharing responsibility for decision making over Role 1 compared to those who were certain [Adjusted OR (95% CI)=3.7 (1.3-10.4)]. **CONCLUSIONS:** Certainty is associated with preferred decision styles. Physicians should ascertain their patients' levels of uncertainty and adjust their input accordingly. **PRACTICE IMPLICATION:** Physicians should ascertain their patients' levels of uncertainty and adjust their input accordingly.

New therapies in the management of hepatitis C virus. Michaels AJ, Nelson DR. *Curr Opin Gastroenterol*. 2010 Mar 10. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/20224396>

PURPOSE OF REVIEW: The present review discusses recent developments in drug discovery for hepatitis C. We are on the verge of a new era with the introduction of direct acting oral agents that will transform the treatment landscape. Both healthcare providers and patients need to stay abreast of these changes that will influence decisions to treat. This article will discuss the most promising up-to-date hepatitis C virus antiviral therapies in clinical investigation as well as the associated clinical trial results. **RECENT FINDINGS:** First generation protease inhibitors will offer higher sustained viral response rates for both naive (70-80%) and treatment-experienced

(40-50%) populations when added to standard pegylated interferon and ribavirin. However, these dramatic gains will be partially offset by new challenges with viral resistance and increased adverse events. **SUMMARY:** There are currently a number of drugs under investigation that target the enzymes involved in hepatitis C virus replication. Year 2011 should bring the approval of the first generation of protease inhibitors that will offer higher cure rates for genotype 1 patients and open the door for the eventual testing of interferon-free regimens.

Role of Cannabinoids in the development of fatty liver (Steatosis). Purohit V, Rapaka R, Shurtleff D. AAPS J. 2010 Mar 5. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/20204561>

Emerging evidence suggests that cannabinoids play an important role in the modulation of fatty liver, which appears to be mediated via activation of cannabinoid receptors. Steatogenic agents such as ethanol and high-fat diet can upregulate the activity of cannabinoid 1 (CB1) receptors via increasing synthesis of endocannabinoids, 2-arachidonoylglycerol, and anandamide. CB1 receptors can also be upregulated by obesity. CB1 receptor activation results in upregulation of lipogenic transcription factor, sterol regulatory element-binding protein 1c and its target enzymes, acetyl-CoA carboxylase-1, and fatty acid synthase and concomitantly, downregulation of carnitine palmitoyltransferase-1. This leads to increased de novo fatty acid synthesis as well as decreased fatty acid oxidation, culminating into the development of fatty liver. High-fat diet, in addition to CB1 receptor activation, appears to activate CB2 receptors that may also contribute to fatty liver. In non-alcoholic fatty liver disease, CB2 receptor activation is associated with the development of fatty liver. Cannabis smoking can increase the severity of fatty liver in hepatitis C patients although the precise mechanism is unknown. As the mechanisms involved in endocannabinoid receptor signaling are being increasingly well understood and the biosynthetic regulatory elements elucidated, these present good opportunity for the pharmaceutical scientists to design drugs to treat liver diseases, including steatosis, based on the cannabinoids, endocannabinoids, and related templates.

Natural anticoagulants can be useful predictors of severity in chronic liver disease. Abdo AA, Sanai FM, Azzam N, et al. Blood Coagul Fibrinolysis. 2010 Mar;21(2):122-7.

<http://www.ncbi.nlm.nih.gov/pubmed/20019598>

Protein S (PS), protein C (PC), and antithrombin (AT) are produced by the liver, and their levels were previously shown to be reduced in chronic as well as acute liver disease. **The aim of this study** was to determine whether measurement of PS, PC, and AT levels in patients would be as good as the commonly used clinical and histological parameters of liver disease in discriminating early and advanced hepatocyte dysfunction. A total of 154 patients were recruited and categorized into five groups: hepatitis B inactive carriers in group 1 (n = 29), nonalcoholic steatohepatitis patients in group 2 (n = 30), chronic hepatitis B patients with elevated liver enzymes in group 3 (n = 29), chronic hepatitis C patients with elevated liver enzymes in group 4 (n = 30), liver cirrhosis patients in group 5 (n = 36). There were significant differences between groups in the levels of PC (P = 0.0001), total PS (P = 0.0001), and free PS (P = 0.0001) and AT (P = 0.0001). These parameters were least affected in the control group, then groups 1 and 2, followed by groups 3 and 4, and most affected in group 5. No differences in these tests were detected between groups 1 and 2 and between groups 3 and 4. Univariate and multivariate analyses showed that free PS was the only predictor of significant inflammation (P = 0.0001), and AT (P = 0.001) and PC (P = 0.003) were the most important factors associated with

advanced fibrosis. Both PS and PC are sensitive markers of liver disease, but PS is a sensitive marker of liver inflammation, whereas PC may be a more sensitive marker for liver fibrosis.

Circulating fibronectin isoforms predict the degree of fibrosis in chronic hepatitis C. Hackl NJ, Bersch C, Feick P, et al. *Scand J Gastroenterol.* 2010 Mar;45(3):349-56.

<http://www.ncbi.nlm.nih.gov/pubmed/20017652>

OBJECTIVE: Hepatic stellate cells only produce fibronectin isoforms in disease states. The isoform-defining domains can be detected in the blood circulation. This study examines whether circulating levels of fibronectin isoforms show a relationship with liver fibrosis on histology in patients with chronic hepatitis C. **MATERIAL AND METHODS:** In a prospective study, 50 patients with chronic hepatitis C who underwent a liver biopsy were compared to 50 matched controls and 35 patients with other liver conditions. **RESULTS:** Circulating levels of the fibronectin isoforms were significantly higher in patients with chronic hepatitis C compared to healthy controls [oncofetal fibronectin (oFN) 2.45 +/- 0.17 versus 1.76 +/- 0.16 mg/l, $P < 0.005$; extra domain-A (EDA) 1.05 +/- 0.06 versus 0.86 +/- 0.06 mg/l, $P < 0.05$; and extra domain-B (EDB) 14.55 +/- 0.74 versus 9.31 +/- 0.58 mg/l, $P < 0.001$], even though total fibronectin was lower (198.9 +/- 3.5 versus 343.6 +/- 14.5 mg/l, $P < 0.001$). A correlation with the fibrosis score was found for both oFN ($r = 0.46$, $P < 0.005$) and EDA ($r = 0.51$, $P < 0.001$). The combination of an elevation in both markers (oFN and EDA) in the upper quartile was associated with a specificity of > 99% for predicting significant fibrosis (stages 2-4) and 95% for predicting advanced fibrosis (stages 3-4). A combination of decreased values in the lowest tertile for both markers had a specificity of 94% for excluding significant fibrosis. Based on these findings, 30% of the patients scheduled for a liver biopsy could be correctly classified as having or not having significant fibrosis. The remainder would have to proceed with a biopsy. **CONCLUSION:** Circulating fibronectin isoforms produced by activated stellate cells represent a viable marker for the presence of significant fibrosis or a lack thereof.