DIAGNOSIS

Clinical and histological features of non-alcoholic fatty liver disease in Hong Kong Chinese

Aliment Pharmacol Ther 2004 Jul 1; 20(1):45-9.

V. W.-S. Wong H. L.-Y. Chan A. Y. Hui K.-F. Chan, C.-T. Liew, F. K.-L. Chan & J. J.-Y. Sung

BACKGROUND: Non-alcoholic fatty liver disease is prevalent in affluent countries and is a cause of cirrhosis and possibly hepatocellular carcinoma.

AIM: To examine the clinical and histological features of biopsy-proven non-alcoholic fatty liver disease and investigate the predictors of severe histological disease in Chinese patients.

METHODS: Electronic records of all patients (n=247) who underwent liver biopsy between 1996 and 2003 in our hospital were retrieved. Patients who had histological features of non-alcoholic fatty liver disease were identified. The demographic, clinical, laboratory and histological (Brunt's criteria) parameters of these patients were analysed.

RESULTS: Forty-two patients had histology-proven non-alcoholic fatty liver disease. The median age was 47 years (range 23-69). All except one patient had features of metabolic syndrome. The median alanine aminotransferase was 93 (range 24-270) IU/L. Thirty-six (85.7%) patients had steatohepatitis and 11 (26.1%) also had fibrosis. Only one patient had stage 3 fibrosis. The presence of diabetes mellitus predicted higher grade steatohepatitis and fibrosis ($P = 0.019$) whereas alanine aminotransferase level had no correlation with histological severity of steatohepatitis. After a median follow-up of 42 months, no patient developed hepatic decompensation.

CONCLUSIONS: Most Chinese patients with non-alcoholic fatty liver disease had features of the metabolic syndrome. Histological activity was generally mild. Diabetes mellitus was the most important predictor of severe histological disease.

Hepatitis C virus-related extra-hepatic disease, aetiopathogenesis and management

Aliment Pharmacol Ther 2004 Jul 15; 20(2):129-41.

J. Medina, L. García-Buey & R. Moreno-Otero

Hepatitis C virus infection is often associated with extra-hepatic manifestations, secondary to the elicitation of autoimmune reactions, generalized deposition of immune complexes and lymphoproliferative disorders. The most clearly established associations are those linking chronic hepatitis C with mixed cryoglobulinaemia (and the related glomerulonephritis and cutaneous vasculitis), as well as with the presence of autoantibodies. Less well-documented disorders include non-Hodgkin's lymphoma, thrombocytopenia, sialadenitis, thyroid disease, lichen planus, porphyria cutanea tarda, rheumatoid disorders and neurological disorders. Extra-hepatic manifestations are most frequent in patients of female sex, advanced age, long-lasting infection and cirrhosis. Optimal treatment strategies should be based on the predominant manifestation of the disease. In the case of autoimmune disorders not clearly attributable to the viral infection, corticosteroids may be the most effective option. Interferon- alone or in combination with ribavirin may be indicated for those disorders related to immune complex deposition, such as mixed cryoglobulinaemia, although relapses of extra-hepatic signs often occur on discontinuation of treatment. In some cases, interferon- may induce or exacerbate some extra-hepatic manifestations.

Monitoring intrahepatic CD8+ T cells by fine-needle aspiration cytology in chronic hepatitis C infection

J Viral Hepat 2004 Jul; 11(4):342-8.

Vrolijk JM, Tang TJ, Kwekkeboom J, Haagmans BL, Herscheid AJ, Kusters JG, Janssen HL, Brouwer JT, Schalm SW.

Infection of the liver with hepatitis C virus (HCV) causes compartmentalization of CD8+ cytotoxic T cells to the site of disease. These cells are thought to be involved in viral clearance during interferon therapy. The repetitive analysis of the intrahepatic immune response is hampered by the difficulty to obtain the intrahepatic T cells. The fine-needle aspiration biopsy (FNAB) technique was evaluated for its use to obtain liver-derived CD8+ T cells in a minimally invasive way. In 26 chronic HCV patients who were evaluated for Peg-interferon and ribavirin combination therapy, pre-treatment FNABs and peripheral blood specimens were obtained simultaneously with liver tissue biopsies, and CD3+ and CD8+ T cells were quantified by immunocytochemistry. The CD8+/CD3+ ratio was significantly higher in the FNABs than in peripheral blood ($P < 0.01$), and similar to those in portal areas in the tissue biopsies. A significant correlation was observed between numbers of CD3+CD8+ T lymphocytes in the FNABs and the numbers of CD8+ cells in the lobular fields or in the portal tracts of the liver tissue biopsies, but not with CD3+CD8+ T lymphocytes in peripheral blood. Finally, the ratio of CD8+/CD3+ T lymphocytes in FNABs was significantly higher in those patients who responded rapidly to therapy when compared with slow responders at 4 weeks of treatment ($P = 0.02$). These findings demonstrate that the intrahepatic T-cell composition is reflected in FNABs, and that the FNAB technique can be used for predicting early virological response to therapy of patients chronically infected with HCV.

Occult hepatitis B viral DNA in liver carcinomas from a region with a low prevalence of chronic hepatitis B infection

J Viral Hepat 2004 Jul; 11(4):297-301.

Kannangai R, Molmenti E, Arrazola L, Klein A, Choti M, Thomas DL, Torbenson M.

Occult hepatitis B is defined by the presence of hepatitis B viral (HBV) DNA in the serum or liver in persons lacking hepatitis B surface antigen (HBsAg) in the serum. A high prevalence of occult HBV has been reported in hepatocellular carcinoma (HCC) from Asia, but little information is available on the prevalence of occult HBV in HCC from regions with a low prevalence of typical chronic hepatitis B infection. In a retrospective study, 19 cases of primary liver cancer were investigated for the presence of occult HBV DNA by amplification of the surface, core, and X gene. In addition, HBV copy numbers were quantitated by real time polymerase chain reaction, genotyped, and samples tested for covalently closed circular HBV DNA, which is a marker of active viral replication. Occult HBV was found in three of 19 cases (16%). Genotyping was successful in two cases, both of which were genotype A. HBV DNA copy numbers were low, all less than 10 copies/micg liver DNA. No closed circular HBV DNA was detected. Thus, in this study occult HBV was of genotype A and was found in a low percentage of cases of HCC and was associated with low tissue HBV DNA copy numbers and no detectable evidence for viral replication.

Occult hepatitis B virus infection in Greek patients with chronic hepatitis C and in patients with diverse nonviral hepatic diseases

J Viral Hepat 2004 Jul; 11(4):358-65.

Georgiadou SP, Zachou K, Rigopoulou E, Liaskos C, Mina P, Gerovasilis F, Makri E, Dalekos GN.

Occult hepatitis B virus (HBV) infection has been reported in patients with chronic hepatitis C who are negative for HBV surface antigen (HBsAg). However, the significance of 'silent' HBV in hepatitis C virus (HCV) infection is unknown. We investigated 540 subjects for the presence of occult HBV in Greek HCV patients, patients with nonviral liver diseases and healthy donors in an attempt to determine the frequency and importance of this phenomenon. One hundred and eighty-seven anti-HCV(+)/HBsAg() patients' sera were investigated for the presence of HBV-DNA by polymerase chain reaction. Two hundred and eighty-two selected blood donors (positive for antibodies to HBV core antigen) and 71 patients with various nonviral hepatic diseases consisted the control groups [both controls were anti-HCV()/HBsAg()]. HBV-DNA was detected in 26.2% of HCV-infected patients vs 8.5% of patients with nonviral diseases (P = 0.003) and 0/282 of donors (P = 0.0000). HBV-DNA was neither associated with HBV markers, nor with the clinical status of HCV and nonHCV patients. Neither epidemiological, histologic and virologic data nor the response to therapy were associated with the HBV-DNA detection. Hence one quarter of HCV-infected patients had occult HBV infection. Similar findings were not found in both control groups. Occult HBV infection in Greek patients with chronic hepatitis C does not seem to modify the progression of chronic liver disease. Further studies of longer duration are needed in order to clarify the role of 'silent' HBV infection in HCV-infected patients.

Mutations in the putative HCV-E2 CD81 binding regions and correlation with cell surface CD81 expression

J Viral Hepat 2004 Jul; 11(4):310-8.

Kronenberger B, Sarrazin C, Hofmann WP, von Wagner M, Herrmann E, Welsch C, Elez R, Ruster B, Piiper A, Zeuzem S.

The hepatitis C virus (HCV) envelope (E)2 protein interacts with the cellular receptor CD81 leading to modulation of B and T cell function. Recently, a higher binding affinity of subtype 1a in comparison with 1b derived E2 proteins for CD81 in vitro was described. The importance of mutations within the putative CD81 binding regions of different HCV geno-/subtypes in correlation with CD81 expression is unknown. In the present study, CD81 expression on blood lymphocytes of patients with chronic hepatitis C infected with different HCV geno-/subtypes were analysed by fluorescence activated cell sorter analyses. In addition, the putative CD81 binding regions on the E2 gene comprising the hypervariable region (HVR)2 were analysed by direct sequencing. CD81 expression on CD8(+) T-lymphocytes from patients infected with subtype 1a (n = 6) was significantly higher in comparison with subtype 1b (n = 12) and 3 (n = 5) infected patients before and during antiviral therapy (P = 0.006; P = 0.021, respectively). Sequencing of the putative CD81 binding regions in the 2 protein comprising the HVR2 (codon 474-495 and 522-552 according to the HCV-1a prototype HCV-H) showed a highly conserved motif within HVR2 for subtype 1a isolates and an overall low number of mutations within the putative CD81 binding regions, whereas numerous mutations were detected for subtype 1b isolates (12.0 vs 23.6%). HCV-3 isolates showed an intermediate number of mutations within the putative binding sites (19.2%; P = 0.022). In conclusion, the highly conserved sequence within HVR2 and putative CD81 binding sites of subtype 1a isolates previously associated with a high CD81 binding affinity in vitro is correlated with high CD81 expression on CD8(+) T-lymphocytes in vivo.

The Role of Serum Zinc and Other Factors on the Prevalence of Muscle Cramps in Non-alcoholic Cirrhotic Patients

Journal of Clinical Gastroenterology July 2004; 8(6): 524-529

Baskol, Mevlut MD; Ozbakir, Omer MD; Coskun, Ramazan MD; Baskol, Gulden MD; Saraymen, Recep PHD; Yucesoy, Mehmet MD

BACKGROUND/AIMS: To determine the prevalence of muscle cramps in patients with liver cirrhosis and to identify factors associated with their development, especially serum zinc.

METHOD: One hundred cirrhotic patients and 85 healthy subjects were enrolled into the study. True muscle cramp was defined as at least 1 painful leg cramp either occurring at rest or strong enough to waken a patient from sleep, occurring at least once a week persisting for a period of greater than 1 year. Creatinine, calcium, magnesium, sodium, potassium, zinc, glucose, alanine aminotransferase, total bilirubin, and albumin levels were detected in sera. Prothrombine time was measured in cirrhotic patients. Presence or absence of ascite was determined by sonography.

RESULTS: True muscle cramps were significantly more common in patients with cirrhosis when compared with the control group (59% vs. 7.1%, respectively, P < 0.001). Cramp (+) cirrhotic patients had older age (49.54 +/- 10.09 vs. 55.54 +/- 7.90, respectively; p: 0.001) and higher Child-Pugh scores (7.56 +/- 2.32 vs. 9.02 +/- 2.55, respectively; p: 0.004) when compared with cramp (-) patients. None of the serum related factors such as creatinine, calcium, magnesium, sodium, potassium, zinc, glucose, alanine aminotransferase, total bilirubin, and albumin levels had any statistically significant contribution to the etiology.

CONCLUSION: Muscle cramps are frequent complication of cirrhosis. Neither biochemical characteristics including decreased serum zinc levels nor the use of diuretics explained the greater prevalence of cramps in patients with cirrhosis. We conclude that the detrimental effect of cirrhosis on muscle fibers may be the major factor.

Prevalence of HBsAg mutants and impact of hepatitis B infant immunisation in four Pacific Island countries

Vaccine 2004 Jul 29; 22(21-22):2791-9.

Basuni AA, Butterworth L, Cooksley G, Locarnini S, Carman WF.

The prevalence rate of hepatitis B virus (HBV) infection in Pacific Island countries is amongst the highest in the world. Hepatitis B immunisation has been incorporated into national programmes at various times, often with erratic supply and coverage, until a regionally co-ordinated programme, which commenced in 1995 ensured adequate supply. The effectiveness of these programmes was recently evaluated in four countries, Vanuatu and Fiji in Melanesia, Tonga in Polynesia and Kiribati in Micronesia. That evaluation established that the programmes had a substantial beneficial impact in preventing chronic hepatitis B infection [Vaccine 18 (2000) 3059]. Several studies of hepatitis B vaccination programmes in endemic countries have identified the potential significance of surface gene mutants as a cause for failure of immunisation. In the study outlined in this paper, we screened infected children and their mothers for the emergence and prevalence of these variants in specimens collected from the four country evaluation. Although the opportunity for the emergence of HBV vaccine escape mutants in these populations was high due to the presence of a considerable amount of the virus in the population and the selection pressure from vaccine use, there were no "a" determinant vaccine escape mutants found. This suggests that vaccine escape variants are not an important cause for failure to prevent HBV transmission in this setting. Other HBsAg variants were detected, but their functional significance remains to be determined. The failure to provide satisfactory protection during such immunisation programmes reflects the need for achieving and sustaining high vaccine coverage, improving the timeliness of doses as well as improving 'cold-chain' support, rather than the selection of vaccine-escape mutants of HBV.

HIV and hepatitis C virus co-infection

Lancet Infect Dis 2004 Jul; 4(7):437-44.

Rockstroh JK, Spengler U.

Since the decline in HIV-related morbidity and mortality after introduction of highly active antiretroviral therapy (HAART) in 1996, liver disease caused by chronic infection with hepatitis C virus (HCV) has become an increasingly important cause of morbidity and mortality among HIV-infected patients infected parenterally with HCV in more developed countries. A third of HIV-infected individuals in Europe and the USA have HCV co-infection. HIV accelerates HCV liver disease especially when HIV-associated immunodeficiency progresses. With the introduction of pegylated interferon in combination with ribavirin, greatly improved treatment options for patients with HIV and HCV co-infection have become available and have led to sustained virological response rates of up to 40%. Furthermore, recent cohort analyses have shown that immune reconstitution induced by HAART can improve the course of hepatitis C leading to a decline in liver-related mortality. However, patients with HCV co-infection are at increased risk of hepatotoxicity from HAART. Owing to the high rates of HIV and HCV co-infection worldwide, new improved treatment strategies and guidelines for the management of co-infection remain a major future goal.

Usefulness of dried blood samples for quantification and molecular characterization of HBV-DNA.

Hepatology 2004 Jul; 40(1):133-9.

Jardi R, Rodriguez-Frias F, Buti M, Schaper M, Valdes A, Martinez M, Esteban R, Guardia J.

The purpose of this study was to assess the use of dried blood spot (DBS) samples for hepatitis B virus (HBV) DNA quantification, HBV genotyping, and detection of G1896A precore mutants and variants in the YMDD polymerase motif. We studied DBS and serum samples from 82 patients with chronic HBV infection (23 hepatitis B e antigen [HBeAg]-positive and 39 HBeAg-negative), 20 HBeAg-inactive carriers, and 15 HBeAg-negative patients under lamivudine therapy (selected from chronic HBV patients). DBS samples consisted of approximately 20 microL of blood applied to 5-mm paper disks. HBV DNA quantification and HBV precore mutant detection were done using real-time polymerase chain reaction, HBV genotyping using restriction fragment length polymorphism, and YMDD variant detection by Inno-lipa assay. DBS and serum results were compared. HBV DNA was detected in a range of 10(2)-10(8) copies/mL, with low intra-assay and inter-assay variation (<10%). Median DBS HBV DNA (copies/mL) was: 3.7 x 10(6) in HBeAg-positive, 6.2 x 10(5) in HBeAg-negative, and 5.5 x 10(2) in inactive carriers (P <.05). HBV DNA was positive in serum (median 5 x 10(3) copies/mL) but negative in DBS for five inactive carriers. The correlation coefficient between HBV DNA concentration in DBS versus serum samples was r(2) = 0.96 (P <.001). The sensitivity of HBV DNA detection in DBS samples was 1 log(10) lower than in serum samples. Concordance between DBS and serum for HBV genotyping, and for precore mutant and YMDD variant detection was optimal. DBS storage for 7 days at room temperature and 21 days at -20 degrees C revealed no decrease in HBV DNA levels or integrity. In conclusion, the DBS sample is useful for HBV DNA quantification, genotyping, and detection of precore mutant and YMDD variants. All four determinations can be completed with a single drop of dried blood.

Longitudinal Study on Mutation Profiles of Core Promoter and Precore Regions of the Hepatitis B Virus Genome in Children.

Pediatr Res. 2004 Jul 7

Ni YH, Chang MH, Hsu HY, Tsuei DJ.

Precore nucleotide 1896 and core promoter mutations may account for hepatitis B e antigen (HBeAg) seroconversion in chronic hepatitis B virus (HBV) infection, yet the mutational profiles of the core promoter are largely unknown in children. An age-matched, case-control study enrolled 110 chronic HBV-infected children, including 55

HBeAg seroconverters and 55 nonseroconverters. Precore and core promoter genes of HBV were sequenced and the serum viral genomes were genotyped from three serial serum samples of the seroconverters and from one serum sample of the nonseroconverters. Higher frequency of A1775G and G1799C mutation rates and lower frequency of A1752G mutation rate were found in the seroconverters. Precore 1896 mutation appeared more in seroconverters than in nonseroconverters (45.5% versus 10.9%; $p < 0.001$). 1762 + 1764 mutation rates were not different between the seroconverters (9.1%) and the nonseroconverters (5.5%). Genotype B was the major type. Genotype C was associated with core promoter 1762 + 1764 mutations in the seroconverter group ($p = 0.023$). The conclusions of this study include the following: 1) mutations of core promoter at nucleotide position 1752, 1775, and 1799 have significant correlations with HBeAg seroconversion; 2) core promoter 1762 + 1764 mutations play a minimal role in HBeAg seroconversion; 3) precore 1896 mutant accounted for half of childhood HBeAg seroconversion; 4) genotype C is associated with 1762 + 1764 mutations during the process of HBeAg seroconversion.

Occult Hepatitis B in HIV-Infected Patients

Acquir Immune Defic Syndr 2004 Jul 1; 36(3):869-75.

Shire NJ, Rouster SD, Rajicic N, Sherman KE.

Prevalence of hepatitis B virus (HBV) markers, including occult HBV, has not been described in diverse cohorts among HIV-infected patients. The objective of this study was to assess prevalence and significance of active and occult HBV infection in an HIV-positive US cohort. A random sample was taken from 2 prospective multicenter treatment intervention cohorts. The sample population (n = 240) was HIV-1 infected and highly active antiretroviral therapy-naive. Prevalence of HBV serologic markers and quantitative HBV DNA were determined. Serum alanine aminotransferase (ALT) levels were measured to evaluate correlates of hepatocyte injury. A total of 64.6% of subjects demonstrated reactivity for any marker of current or past HBV infection or prior vaccination. Chronic HBV infection characterized by hepatitis B surface antigen (HBsAg) reactivity was present in 7.1% while 15.8% exhibited HB anticore IgG only. Approximately 10% of the latter group was HBV DNA positive by a polymerase chain reaction-based assay. Only patients with a serologic pattern of HBsAg or HB anticore alone reactivity had HBV DNA. Occult HBV was observed in approximately 10% of HIV-infected patients with HB anticore IgG antibody in a geographically representative national cohort. Though viral titers and serum ALT levels were low, screening of this subset of HIV-infected patients may have implications in terms of antiretroviral therapy and risk of immune reconstitution-associated flares.

The evolving role of liver biopsy

Alimentary Pharmacology & Therapeutics 2004 Aug; 20 (4):249-259.

M. S. Campbell, K. R. Reddy

Liver biopsy is traditionally the 'gold standard' for the evaluation of liver diseases. There are several situations in which its role is being challenged. In hepatitis C, liver biopsy helps assess prognosis and treatment candidacy. An important exception is genotype 2 or 3 because treatment is more likely to succeed and therapy is relatively short in duration. For hepatitis B, liver biopsy gives some prognostic information, but serologic tests and hepatic biochemical tests are the primary determinants of treatment candidacy. Non-alcoholic fatty liver disease can be accurately diagnosed without a liver biopsy and, furthermore, there are no specific therapies available. The role of liver biopsy to assess methotrexate-associated hepatotoxicity remains controversial. Finally, patients with focal liver lesions usually do not require biopsy and, in the case of hepatocellular carcinoma, biopsy carries a risk of needle-track seeding. In short, the need for liver biopsy depends on the specific situation and should be performed when there is sufficient uncertainty about diagnosis, severity of disease, prognosis, and treatment decisions.

Assessment of correlation between serum titers of hepatitis c virus and severity of liver disease.

World J Gastroenterol 2004 Aug 15; 10(16):2409-11.

Anand BS, Velez M.

AIM: The significance of hepatitis C virus (HCV) serum titers has been examined in several clinical situations. There is much evidence that patients with a lower viral load have better response rates to anti-viral therapy compared to those with higher levels. Moreover, a direct association has been observed between serum titers of HCV and transmission rates of the virus. The aim of the present study was to determine if there was any correlation between HCV viral load and the severity of liver disease.

METHODS: Fifty patients with HCV infection were included in the study. These comprised of 34 subjects with a history of alcohol use and 16 non-alcoholics. Quantitative serum HCV RNA assay was carried out using the branched DNA (bDNA) technique. Linear regression analysis was performed between serum viral titers and liver tests. In addition, for the purpose of comparison, the subjects were divided into two groups: those with low viral titers (<=50 genome mEq/mL) and high titers (>50 mEq/mL).

RESULTS: All subjects were men, with a mean+/-SD age of 47+/-7.8 years. The mean HCV RNA level in the blood was 76.3X10(5) +/-109.1 genome equivalents/mL. There was no correlation between HCV RNA levels and age of the patients (r = 0.181), and the history or amount (g/d) of alcohol consumption (r = 0.07). Furthermore, no correlation was observed between serum HCV RNA levels and the severity of liver disease as judged by the values of serum albumin (r = 0.175), bilirubin (r = 0.217), ALT (r = 0.06) and AST (r = 0.004) levels. Similarly, no significant difference was observed between patients with low viral titers and high titers with respect to any of the parameters.

CONCLUSION: Our results indicate that the severity of liver disease is independent of serum levels of hepatitis C virus. These findings are important since they have a direct impact on the current debate regarding the role of direct cytopathic effect of hepatitis C virus versus immune-mediated injury in the pathogenesis of HCV-related liver damage.

Clinical evaluation of the digene hybrid capture II test and the COBAS AMPLICOR monitor test for determination of hepatitis B virus DNA levels

J Clin Microbiol 2004 Aug; 42(8):3513-7.

Yuan HJ, Yuen MF, Wong DK, Sum SS, Lai CL.

The measurement of hepatitis B virus (HBV) DNA is important for the assessment of liver disease and treatment efficacy. Most commercially available assays for the determination of HBV DNA levels have limited linear ranges. This study was performed to evaluate the clinical performance of the Digene Hybrid Capture II (Digene HC II assay) and the COBAS AMPLICOR Monitor test (COBAS-AM assay), with special emphasis on anti-HBV e antigen (HBeAg)-positive patients with low HBV DNA levels. A total of 425 Chinese patients with chronic hepatitis B were recruited. A total of 107 patients were HBeAg positive, and 318 patients were HBeAg negative. The Digene HC II assay and the COBAS-AM assay had similar intra-assay and interassay variabilities. A total of 264 patients (62.1%) had HBV DNA levels undetectable by the Digene HC II assay, and 47 patients (11.1%) had HBV DNA levels undetectable by the COBAS-AM assay ($P < 0.001$). For the 161 patients with HBV DNA levels detectable by the Digene HC II assay, the HBV DNA levels obtained by the Digene HC II assay and by the COBAS-AM assay showed an excellent correlation ($r = 0.95$; $P < 0.001$). The linear ranges of the Digene HC II assay and the COBAS-AM assay marginally overlapped. Before HBV DNA levels could be determined by the COBAS-AM assay, predilution had to be performed for 158 of 161 patients (98.1%) with HBV DNA levels detectable by the Digene HC II assay and for 10 of 264 patients (3.8%) with HBV DNA levels undetectable by the Digene HC II assay. The cost for assaying each serum sample by using different strategies was calculated. The COBAS-AM assay was more sensitive than the Digene HC II assay and more suitable for monitoring low levels of HBV viremia.

Genotype prevalence, viral load and outcome of hepatitis B virus precore mutant infection in stable patients and in patients after liver transplantation

Clin Transplant 2004 Aug; 18(4):415-22.

Ben-Ari Z, Ashur Y, Daudi N, Shmilovitz-Wiess H, Brown M, Sulkes J, Klein A, Mor E, Tur-Kaspa R, Shouval D.

OBJECTIVE: The precore mutant is detectable in most Israeli patients with persistent hepatitis B virus (HBV) infection. The aim of this study was to determine the prevalence of HBV genotypes, viral load and outcome of precore mutant infection in stable patients and in patients after liver transplantation.

METHODS: The prevalence of HBV genotype and viral load were investigated in 81 patients with HBV precore mutant infection. Of these, 50 patients (40 males, 10 females; mean age 43.4 +/- 11.0 yr) underwent liver transplantation and were serum HBV DNA-negative by hybridization at the time of transplantation. Patients received long-term HBV immunoprophylaxis and immunosuppression, and lamivudine in cases of graft HBV recurrence. The remaining 31 patients were stable, with serum anti-HBe-positivity. Genotypes were tested by restriction fragment length polymorphism of an S gene amplicon. Precore mutations were studied with an INNO-LiPA probe assay.

RESULTS: Follow-up was 46.6 +/- 37.7 months. Most of the transplanted group was of Middle Eastern origin (53.6%); the remainder were from Eastern Europe (21.4%), Western Europe and the USA (10.8%), Africa (7.1%), and Asia (7.1%). In the transplanted group, the pre-transplant HBV genotype D was the most prevalent (96%), while genotype A was found in only 4%. Eleven patients (22%) developed recurrent HBV infection post-transplantation. There were no differences in genotype distribution between patients with graft reinfection or lamivudine resistance and patients without recurrence. Mean viral load at recurrence was 148.4 x 10(6) +/- 60.4 x 10(6) copies/mL. The stable group had a similar origin and HBV genotype prevalence, but a lower mean viral load of 12.4 x 10(6) +/- 29.4 x 10(6) copies/mL ($p = 0.007$). The prevalence of mutations at the precore region and codon 28 was similar in both groups.

CONCLUSIONS: The chronic precore mutant HBV-infected patients were characterized as follows: (i) genotype D was the most frequent genotype, (ii) the HBV genotype distribution was similar in patients with stable infection and after liver transplantation, (iii) viral load at recurrence was significantly higher than in stable infection, and (iv) HBV genotype was unrelated to the development of recurrence or lamivudine resistance in the tested population.

Distribution of HBV genotypes and mutants among hepatitis B infected patients from northern Poland.

Int J Mol Med 2004 Aug; 14(2):301-4.

Bielawski KP, Dybikowska A, Lisowska-Charmuszko U, Stalke P, Gregorowicz K, Trocha H, Podhajska A.

Hepatitis B virus (HBV) infection is one of the major global epidemiological problems. The aim of our study was to determine the distribution of HBV genotypes in Poland since the data concerning the spread of HBV viruses in the central-eastern region of Europe is still very limited. HBV DNA was extracted from 58 serum samples. To quantify the level of HBV DNA the Roche Amplicor HBV Monitor Assay was used. To genotype and assign HBV subtypes DNA sequencing methods were performed. The HBV virus from 43 serum samples from hepatitis B infected patients was genotype A (74.1%), 12 cases had genotype D (20.7%), and 3 had the rare in Europe genotype F (5.2%). Prediction of HBV serological subtypes based on HBsAg sequencing showed almost 100% occurrence of subtype adw2 in the group of genotype A samples, three different subtypes in genotype D (ayw2, ayw3, and ayw4), and equal distribution of subtype adw4q(-) in all 3 cases of genotype F, also the most prevalent subtype in the Amerindians. Our results coincide with the general European HBV prevalence. However, HBV genotype F, which is not a common genotype in European countries, was detected and so was relatively high occurrence of genotype D, which may reflect historical and ethnical migration events in Poland in the past.

Genotype and phylogenetic characterization of hepatitis B virus among multi-ethnic cohort in Hawaii

World J Gastroenterol 2004 Aug 1; 10(15):2218-22.

Sakurai M, Sugauchi F, Tsai N, Suzuki S, Hasegawa I, Fujiwara K, Orito E, Ueda R, Mizokami M.

AIM: Hepatitis B virus (HBV) genomes in carriers from Hawaii have not been evaluated previously. The aim of the present study was to evaluate the distribution of HBV genotypes and their clinical relevance in Hawaii.

METHODS: Genotyping of HBV among 61 multi-ethnic carriers in Hawaii was performed by genetic methods. Three complete genomes and 61 core promoter/precore regions of HBV were sequenced directly.

RESULTS: HBV genotype distribution among the 61 carriers was 23.0% for genotype A, 14.7% for genotype B and 62.3% for genotype C. Genotypes A, B and C were obtained from the carriers whose ethnicities were Filipino and Caucasian, Southeast Asian, and various Asian and Micronesian, respectively. All cases of genotype B were composed of recombinant strains with genotype C in the precore plus core region named genotype Ba. HBeAg was detected more frequently in genotype C than in genotype B (68.4% vs 33.3%, $P<0.05$) and basal core promoter (BCP) mutation (T1762/A1764) was more frequently found in genotype C than in genotype B. Twelve of the 38 genotype C strains possessed C at nucleotide (nt) position 1858 (C-1858). However there was no significant difference in clinical characteristics between C-1858 and T-1858 variants. Based on complete genome sequences, phylogenetic analysis revealed one patient of Micronesian ethnicity as having C-1858 clustered with two isolates from Polynesia with T-1858. In addition, two strains from Asian ethnicities were clustered with known isolates in carriers from Southeast Asia.

CONCLUSION: Genotypes A, B and C are predominant types among multi-ethnic HBV carriers in Hawaii, and distribution of HBV genotypes is dependent on the ethnic background of the carriers in Hawaii.

Cloning and expression of surface antigens from occult chronic hepatitis B virus infections and their recognition by commercial detection assays

J Med Virol 2004 Aug; 73(4):508-15.

Jeantet D, Chemin I, Mandrand B, Tran A, Zoulim F, Merle P, Trepo C, Kay A.

Occult hepatitis B virus (HBV) infections show little or no serological markers of viral infection, including the absence of hepatitis B surface antigen (HBsAg) which is the main marker of ongoing HBV infection. Such infections can be important in the context of blood and/or organ donations. To study whether mutations contribute to HBsAg seronegativity, S gene sequences from such patients were amplified and cloned. Sequencing revealed 12 clones from seven different patients which contained potentially important mutations. The sequences were subcloned into an expression vector and mutant HBsAgs were expressed in cell culture. The capacity of three HBsAg detection assays to recognise the mutant HBsAgs was studied. Three categories were found: mutant HBsAgs that are not recognised by the assays, those that are recognised as well as wild-type (WT) antigen and an intermediate category where detection of the mutant HBsAgs is reduced with respect to WT. Most of the isolates fall into the second category. Mutations can therefore contribute to HBsAg seronegativity in occult HBV infections, but in most cases the explanation is probably the low level of viral replication.

Detection of hepatitis D virus by cDNA microarray method

Hepatobiliary Pancreat Dis Int 2004 Aug; 3(3):423-7.

Sun ZH, Zheng WL, Zhang B, Lu L, Mao XD, Shi R, Ma WL.

BACKGROUND: Viral hepatitis is considered a major public health problem in most areas of the world. In acute and chronic infections, hepatitis D virus (HDV) infection often leads to a more severe disease.This study was designed to prepare microarrays for HDV detection.

METHODS: The specific primers of PCR were designed according to the conserved region of HDV. The cDNA microarrays were prepared by spotting PCR products onto the surface of glass slides by robotics. Restriction display PCR (RD-PCR) was used to label the samples.

RESULTS: Sequences were aligned, and the results showed that the products of PCR amplification were the specific gene fragments of HDV. Hybridizing signals on gene chip showed the specificity and sensitivity in detecting HDV were satisfactory.

CONCLUSION: Using PCR amplified products to construct gene chips for clinical diagnosis of HDV is a quick, simple and effective method.

Detection of YMDD Motif Mutants by Oligonucleotide Chips in Lamivudine-Untreated Patients with Chronic Hepatitis B Virus Infection

J Korean Med Sci 2004 Aug; 19(4):541-6.

Heo J, Cho M, Kim HH, Shin YM, Jang HJ, Park HK, Kim CM, Kim GH, Kang DH, Song GA, Yang US.

Lamivudine, a nucleoside analogue, has been used widely as an effective antiviral agent for the treatment of patients with chronic hepatitis B virus (HBV) infection. However, the YMDD motif mutation of HBV polymerase resistant to lamivudine occurs very frequently after long term therapy. We developed an oligonucleotide chip for the detection of YMDD motif mutants resistant to lamivudine and investigated the prevalence of the mutants in patients with chronic HBV infection who had not been treated by lamivudine before. Forty patients who had not been treated with lamivudine were included in this study. Serum samples were tested by the oligonucleotide chips designed for detection of wild-type YMDD motif, M552V and M552I. Samples were confirmed by restriction fragment length polymorphism (RFLP) and direct sequencing. M552I mutants were detected by the oligonucleotide chips in 7.5% (3/40) of chronic HBV infected patients (2 chronic hepatitis and 1 cirrhosis). The results were in accordance with those of RFLP. YMDD motif mutants occur as natural genome variabilities in patients with chronic HBV infection who had not been treated with lamivudine before. Oligonucleotide chip technology is a reliable and useful diagnostic tool for the detection of mutants resistant to antiviral therapy in chronic HBV infection.

Hepatitis C virus NS5A: tales of a promiscuous protein

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Macdonald A, Harris M.

The non-structural 5A (NS5A) protein of hepatitis C virus (HCV) has been the subject of intensive research over the last decade. It is generally accepted that NS5A is a pleiotropic protein with key roles in both viral RNA replication and modulation of the physiology of the host cell. Our understanding of the role of NS5A in the virus life cycle has been hampered by the lack of a robust in vitro system for the study of HCV replication, although the recent development of the subgenomic replicon has at least allowed us to begin to dissect the involvement of NS5A in the process of viral RNA replication. Early studies into the effects of NS5A on cell physiology relied on expression of NS5A either alone or in the context of other non-structural proteins; the advent of the replicon system has allowed the extrapolation of these studies to a more physiologically relevant cellular context. Despite recent progress, this field is controversial, and there is much work to be accomplished before we fully understand the many functions of this protein. In this article, the current

state of our knowledge of NS5A, discussing in detail its direct involvement in virus replication, together with its role in modulating the cellular environment to favour virus replication and persistence, are reviewed. The effects of NS5A on interferon signalling, and the regulation of cell growth and apoptosis are highlighted, demonstrating that this protein is indeed of critical importance for HCV and is worthy of further investigation.

Liver iron deposits in hepatitis B patients: Association with severity of liver disease but not with hemochromatosis gene mutations

J Gastroenterol Hepatol 2004 Sep; 19(9):1036-1041.

Martinelli AL, Filho AB, Franco RF, Tavella MH, Ramalho LN, Zucoloto S, Rodrigues SS, Zago MA.

Background and Aims: Iron deposits in the liver and abnormalities in serum iron biochemistry are frequently observed in patients with chronic liver diseases, but data for patients with hepatitis B virus (HBV) infection are scarce. Moreover, the role of HFE mutations in iron deposits in this condition remains unknown. The aim of the present study was to determine the prevalence of serum iron biochemical abnormalities and iron deposits in the liver of chronic HBV patients, and to evaluate the consequences for the activity and severity of liver disease. Additionally, we studied the role of HFE gene mutations in iron deposits. Methods: Eighty-one male non-cirrhotic HBV patients were studied. Serum iron biochemistry, liver enzymes and C282Y/H63D mutations were investigated. Liver biopsies were scored for necroinflammatory activity (histological activity index [HAI]), fibrosis and iron deposits.

Results: Elevated transferrin saturation (TS) was found in 27.1% of patients and liver iron deposits in 48.7%; these deposits were mild in 68.4% and moderate in 31.6%. Patients with liver iron deposits exhibited significantly higher scores for HAI and fibrosis than those without iron deposits. HFE mutations were identified in 23.4% of patients (14 H63D heterozygotes, four H63D homozygotes, one compound mutation). No difference in the prevalence of C282Y and H63D mutations was observed between HBV patients (1.2% and 23.4%, respectively) and the general population (4.1% and 27.8%, respectively). No association was detected between HFE mutations and elevated TS or liver iron deposits.

Conclusions: Elevated TS and liver iron deposits were frequent in non-cirrhotic HBV patients. Iron deposits were mainly mild and associated with higher activity and severity of liver disease, but not with HFE mutations.

Molecular epidemiology of hepatitis B virus infections in Denmark

J Clin Virol 2004 Sep; 31(1):46-52.

Fisker N, Pedersen C, Lange M, Nguyen NT, Nguyen KT, Georgsen J, Christensen PB.

Background: Denmark has a low incidence of acute hepatitis B (HBV) infections but the impact of an increasing number of immigrants with chronic HBV infection on HBV transmission is unknown.

Objectives: To characterise individuals with chronic and acute HBV infection in a defined region and to examine the importance of different risk groups for the current HBV transmission.

Methods: During 2000-2001 all consecutive HBV infected individuals routinely diagnosed through the regional HBV serology laboratory in the County of Funen were classified according to ethnicity, presumed route of transmission and stage of infection based on clinical data mainly supplied by the requesting physician. HBV DNA was sequenced and subjected to phylogenetic analysis

Results: Of 309 identified cases, 91 (29%) were classified as acute infection. HBV DNA sequencing was possible in 54 (59%) of these cases. Phylogenetic analysis showed that HBV isolated from injecting drug users (IDUs) was identical or closely related. Among acute cases acquired in Denmark 89% (74/83) were seen in IDUs (65) or in individuals presumably exposed to IDUs (nine) and phylogenetic analysis corroborated the assumption of IDU related transmission in every case with available sequence data. Among 83 ethnic Danes who acquired their HBV infection in Denmark, no new cases of transmission from immigrants were detected.

Conclusion: Injecting drug use was the single most important factor for hepatitis B transmission in Denmark. The current Danish vaccination strategy is unable to protect IDUs from HBV infection and IDUs pose a greater risk of HBV transmission to the general population than immigrants.

Simultaneous quantitation and genotyping of hepatitis B virus by real-time PCR and melting curve analysis

J Virol Methods 2004 Sep 15; 120(2):131-40.

Payungporn S, Tangkijvanich P, Jantaradsamee P, Theamboonlers A, Poovorawan Y.

Hepatitis B virus (HBV) genotype and HBV DNA levels have been implicated in clinical evaluation and prognosis of patients with chronic HBV infection. The aim of the present study was to develop a rapid and sensitive method for simultaneous HBV DNA quantitation and differentiation between HBV genotypes B and C in a single-step reaction by real-time PCR and melting curve analysis using SYBR Green I fluorescent dye. The genotypes obtained by this method were compared with those examined by PCR-RFLP and direct sequencing on 52 serum samples of patients with chronic HBV infection. Using the results obtained by direct sequencing and phylogenetic analysis as the reference, the accuracy of HBV genotyping by PCR-RFLP and melting curve analysis was 90.38 and 92.31%, respectively. The geometric mean of HBV DNA levels was [Formula: see text], [Formula: see text], [Formula: see text] and [Formula: see text] copies/microl in asymptomatic carriers, patients with chronic hepatitis, cirrhosis and hepatocellular carcinoma, respectively. It is concluded that this method has the advantages of rapidity, reproducibility and accuracy, which would be feasible and attractive for large-scale analysis, particularly in regions where HBV genotypes B and C are prevalent.

Correlation of serum aminotransferases with HCV RNA levels and histological findings in patients with chronic hepatitis C: the role of serum aspartate transaminase in the evaluation of disease progression

Eur J Gastroenterol Hepatol 2004 Sep; 16(9):891-896.

Zechini B, Pasquazzi C, Aceti A.

OBJECTIVES: To investigate whether HCV RNA levels can be considered to be predictors of hepatocellular injury in patients with chronic hepatitis C, and whether aminotransferase levels are markers of liver damage.

METHODS: We performed a retrospective study on 112 patients with chronic hepatitis C. For each patient, we considered the baseline alanine aminotransferase (ALT) and serum aspartate transaminase (AST) levels, baseline HCV RNA, HCV genotype, histological evaluation and the mean aminotransferase levels measured in the 6 months following liver biopsy.

RESULTS: We found a statistically significant correlation between HCV RNA and aminotransferase levels measured during the follow-up (AST: r = 0.24, P = 0.01; ALT: r = 0.27, P = 0.004). We also observed a statistically significant correlation between HCV RNA levels and histological activity index (HAI) (r = 0.25, P = 0.008), as well as between the HAI and both baseline AST (r = 0.34, P = 0.0002) and ALT levels (r = 0.23, P = 0.01). These findings were confirmed by the mean aminotransferase values during follow-up. In the regression analysis, the fibrosis score was significantly and independently associated with baseline AST and ALT values.

CONCLUSIONS: Our results demonstrate a statistically significant correlation of aminotransferase values with the histological parameters, and an even stronger correlation with the AST values. Our study therefore suggests that aminotransferase values, especially AST, may correlate with liver damage.

Prevalence of Production of Virus-Specific Interferon- gamma among Seronegative Hepatitis C-Resistant Subjects Reporting Injection Drug Use

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Freeman AJ, Ffrench RA, Post JJ, Harvey CE, Gilmour SJ, White PA, Marinos G, Van Beek I, Rawlinson WD, Lloyd AR.

This report describes subjects who were highly likely to have been repeatedly exposed to hepatitis C virus (HCV) through injection drug use and who remained negative for anti-HCV antibody. Production of virus-specific interferon-gamma by peripheral blood mononuclear cells was seen in the majority of subjects (72%) and was associated with higher-risk behavior. For 92% of the subjects, results of recombinant immunoblot assays demonstrated faint bands against nonstructural proteins. The immune responses described are likely to have been primed and maintained by episodes of subclinical infection without classic seroconversion and may indicate a hepatitis C-resistant phenotype. Vaccine strategies to mimic this response may provide protection against persistent HCV infection.

A vaccinia replication system for producing recombinant hepatitis C virus

World J Gastroenterol 2004 Sep 15; 10(18):2670-4.

Wu YS, Feng Y, Dong WQ, Zhang YM, Li M.

AIM: To develop a cell culture system capable of producing high titer hepatitis C virus (HCV) stocks with recombinant vaccinia viruses as helpers.

METHODS: Two plasmids were used for the generation of recombinant HCV: one containing the full-length HCV cDNA cloned between T7 promoter and T7 terminator of pOCUS-T7 vector, and the other containing the HCV polyprotein open reading frame (ORF) directly linked to a vaccinia late promoter in PSC59. These two plasmids were co-transfected into BHK (21) cells, which were then infected with vTF7-3 recombinant vaccinia helper viruses.

RESULTS: After 5 d of incubation, approximately 3.6X10(7) copies of HCV RNA were present per milliliter of cell culture supernatant, as detected by fluorescence quantitative RT-PCR (FQ-PCR). The yield of recombinant HCV using this cell system increased 100- to 1 000- fold compared to in vitro- transcribed HCV genomic RNA or selective subgenomic HCV RNA molecule method.

CONCLUSION: This cell culture system is capable of producing high titer recombinant HCV.

Establishment and assessment of two methods for quantitative detection of serum duck hepatitis B virus DNA

World J Gastroenterol 2004 Sep 15; 10(18):2666-9.

AIM: To establish and assess the methods for quantitative detection of serum duck hepatitis B virus (DHBV) DNA by quantitative membrane hybridization using DHBV DNA probe labeled directly with alkaline phosphatase and fluorescence quantitative PCR (qPCR).

METHODS: Probes of DHBV DNA labeled directly with alkaline phosphatase and chemiluminescent substrate CDP-star were used in this assay. DHBV DNA was detected by autoradiography, and then scanned by DNA dot-blot. In addition, three primers derived from DHBV DNA S gene were designed. Semi-nested primer was labeled by AmpliSensor. Standard curve of the positive standards of DHBV DNA was established after asymmetric preamplification, semi-nested amplification and on-line detection. Results from 100 samples detected separately by alkaline phosphatase direct-labeled DHBV DNA probe with dot-blot hybridization and digoxigenin-labeled DHBV DNA probe hybridization. Seventy samples of duck serum were tested by fluorescent qPCR and digoxigenin-labeled DHBV DNA probe in dot-blot hybridization assay and the correlation of results was analysed.

RESULTS: Sensitivity of alkaline phosphatase direct-labeled DHBV DNA probe was 10 pg. The coincidence was 100% compared with digoxigenin-labeled DHBV DNA probe assay. After 30 cycles, amplification products showed two bands of about 180 bp and 70 bp by 20 g/L agarose gel electrophoresis. Concentration of amplification products was in direct proportion to the initial concentration of positive standards. The detection index was in direct proportion to the quantity of amplification products accumulated in the current cycle. The initial concentration of positive standards was in inverse proportion to the number of cycles needed for enough quantities of amplification products. Correlation coefficient of the results was (0.97, P<0.01) between fluorescent qPCR and dot-blot hybridization.

CONCLUSION: Alkaline phosphatase direct-labeled DHBV DNA probe in dot-blot hybridization and fluorescent qPCR can be used as valuable means to quantify DHBV DNA in serum.

Should a liver biopsy be done in patients with subclinical chronically elevated transaminases?

Eur J Gastroenterol Hepatol 2004 Sep; 16(9):879-883.

De Ledinghen V, Combes M, Trouette H, Winnock M, Amouretti M, De Mascarel A, Couzigou P.

In 10% of the patients with chronic abnormal alanine aminotransferase (ALT) levels no cause is found. The prognosis of this liver disease, the increased risk of liver fibrosis regardless of the types of histological lesions and the need for a liver biopsy are unknown. Nearly 50% of these cases are explained by non-alcoholic steatohepatitis (NASH). The aim of this study was to evaluate, in patients with accidentally detected chronically elevated ALT levels, the prevalence of fibrosis and NASH, and the clinical and biological factors associated with each entity. Retrospectively, 67 patients (mean age, 46.6 +/- 12.1 years; 45 males) were included. All patients had a liver biopsy and were hepatitis B virus, hepatitis C virus, human immunodeficiency virus seronegative without alcohol, drug, autoimmune or genetically induced liver disease, with ALT > N (the upper limit of normal). NASH was evaluated according to necroinflammatory lesions and fibrosis. Fibrosis was evaluated according to the METAVIR score. Statistical analyses were performed using Student's t test, the Mann-Whitney rank-sum test and the chi-square test. Fibrosis scores were: F0, 37.3%; F1, 32.8%; F2, 26.9%; F3, 1.5%; and F4, 1.5%. NASH was absent in 59.7% and present in 40.3%. Significant differences were observed between F < 2 and F >/= 2 fibrosis patients for aspartate aminotransferase (AST) and ALT and between patients with NASH or without for body mass index. Overall, the risk of F >/= 2 fibrosis was increased in patients with AST > N, ALT > 2N or AST > N and ALT > 2N. The prevalence of F >/= 2 fibrosis and NASH in patients with unexplained chronic abnormal ALT are 30% and 40%, respectively. Since the risk of F >/= 2 fibrosis is significantly increased in patients with AST > N and/or ALT > 2N, liver biopsy should be performed only in patients with AST > N or ALT > 2N.

Hepatitis C virus E2 has three immunogenic domains containing conformational epitopes with distinct properties and biological functions

J Virol 2004 Sep; 78(17):9224-32.

Keck ZY, Op De Beeck A, Hadlock KG, Xia J, Li TK, Dubuisson J, Foung SK.

Mechanisms of virion attachment, interaction with its receptor, and cell entry are poorly understood for hepatitis C virus (HCV) because of a lack of an efficient and reliable in vitro system for virus propagation. Infectious HCV retroviral pseudotype particles (HCVpp) were recently shown to express native E1E2 glycoproteins, as defined in part by HCV human monoclonal antibodies (HMAbs) to conformational epitopes on E2, and some of these antibodies block HCVpp infection (A. Op De Beeck, C. Voisset, B. Bartosch, Y. Ciczora, L. Cocquerel, Z. Y. Keck, S. Foung, F. L. Cosset, and J. Dubuisson, J. Virol. 78:2994-3002, 2004). Why some HMAbs are neutralizing and others are nonneutralizing is looked at in this report by a series of studies to determine the expression of their epitopes on E2 associated with HCVpp and the role of antibody binding affinity. Antibody cross-competition defined three E2 immunogenic domains with neutralizing HMAbs restricted to two domains that were also able to block E2 interaction with CD81, a putative receptor for HCV. HCVpp immunoprecipitation showed that neutralizing and nonneutralizing domains are expressed on E2 associated with HCVpp, and affinity studies found moderate-to-high-affinity antibodies in all domains. These findings support the perspective that HCV-specific epitopes are responsible

for functional steps in virus infection, with specific antibodies blocking distinct steps of virus attachment and entry, rather than the perspective that virus neutralization correlates with increased antibody binding to any virion surface site, independent of the epitope recognized by the antibody. Segregation of virus neutralization and sensitivity to low pH to specific regions supports a model of HCV E2 immunogenic domains similar to the antigenic structural and functional domains of other flavivirus envelope E glycoproteins.

Mutations of the interferon sensitivity-determining region (ISDR) correlate with the complexity of hypervariable region (HVR)-1 in the Japanese variant of hepatitis C virus (HCV) type 1b

J Med Virol 2004 Sep; 74(1):54-61.

Nakano I, Fukuda Y, Katano Y, Toyoda H, Hayashi K, Kumada T, Nakano S.

Hepatitis C virus (HCV) genotype 1b comprises mainly two subtypes in Japan, each named for its geographic prevalence (Japan-specific, J type; worldwide, W type). Because the newly identified subtypes have not been fully characterized, the present study directed this issue from virological viewpoints such as hypervariable region (HVR)-1 as well as interferon (IFN) sensitivity-determining region (ISDR). Fifty chronic hepatitis patients with HCV 1b (31 men and 19 women; mean age 50.5 years) were enrolled, and J/W type was determined according to envelope 1 (E1) sequence as described previously (23 J type and 27 W type). Correlations between age, number of HVR-1 clones, HVR-1 diversity, and ISDR mutations were analyzed in J and W type patients independently. In addition, the sequences of the three HCV regions obtained for the determination of the above genetic factors were studied phylogenetically. The number of HVR-1 clones was significantly higher for J type in comparison with W type ($P = 0.044$). In the J type-infected patients, the ISDR mutation number was correlated inversely with HVR-1 clone number ($P = 0.0001$, $r = -0.734$) and HVR-1 diversity ($P = 0.0001$, $r = -0.722$). However, this correlation was not observed in the W type patients. W type patients showed a significant correlation between age and HVR-1 clone number ($P = 0.015$, $r = 0.462$). Phylogenetic study revealed that the nonstructural (NS) 5A sequence, which is obtained for ISDR type determination, can distinguish between J and W types. The inverse correlation in J type patients between ISDR mutations and HVR-1 complexity may explain the usefulness of the ISDR for prediction of IFN response only in Japanese patients. This suggests that the ISDR is not directly related to IFN responsiveness, but the degree of HVR-1 complexity may be more important.