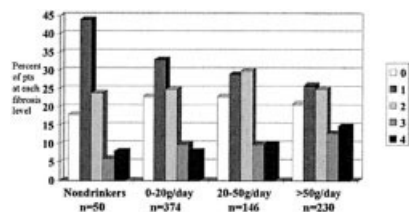


HEPATOLOGY HIGHLIGHTS

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You Can Lead a Patient to Alcohol, But You Can't Make Him Fibrose



The study of Monto et al. gives a sobering account of the effects of various amounts of alcohol intake on hepatitis C-related fibrosis. At issue is

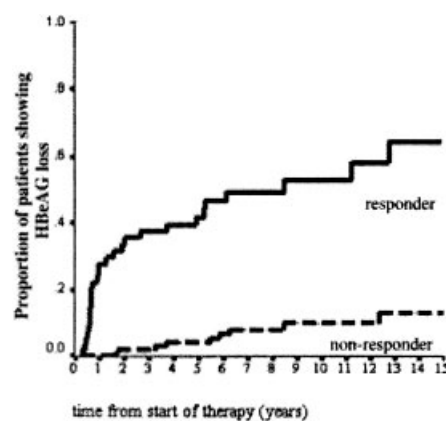
the compounding effect of alcohol and HCV and the practical concern of how much alcohol an HCV-infected patient can safely consume. The literature is replete with studies that uniformly show that high alcohol intake (>50-80 g/day) greatly exacerbates fibrosis progression and cirrhosis development in patients with HCV infection. The net outcome has always been much more than additive. Few studies, however, have systematically examined the effects of lesser quantities of alcohol, and in those that have, the results have been conflicting. Monto et al. performed a careful estimation of lifetime alcohol intake and derived an average consumption in grams per day based on the calculation that one drink equals 10 grams of pure ethanol. The cohort was then divided into light (0-20 g/day), moderate (20-50 g/day) and heavy (>50 g/day) consumption. The mean alcohol consumption in the cohort was 41.6 g/day, but it was not normally distributed so that the median was 17.4 g/d; 29% consumed >50 g/day. Interestingly, alcohol intake did not correlate with the ALT or the inflammatory score on liver biopsy, suggesting that alcohol does not exert its fibrotic effects by enhancing inflammation. Alcohol intake was associated with a stepwise increase in mean fibrosis, and if alcohol use was dichotomized into ≥ 50 g/day versus <50 g/day, there was a significant difference ($P = .01$) between the groups. If dichotomized at ≥ 80 g/day, the difference was even greater ($P = .006$), but no significant difference in fibrosis was found if dichotomization was performed at lower levels of intake. In a multivariate analysis, histologic inflammation, serum ALT, and patient age were independent predictors of fibrosis, but alcohol was not, though it was close ($P = .06$). Importantly, at every alcohol level, there was a broad range of fibrosis outcomes, and even among heavy drinkers, 47% had stage 0 or stage 1 fibrosis (Fig.). Just as in persons not infected with HCV, there appears to be a range of susceptibility to the fibrotic effects of alcohol.

Because there was a stepwise increase in the odds ratio for

fibrosis with each level of alcohol intake, the authors conclude that even light alcohol intake may be playing a role in fibrosis development and that their study size of 800 "... may be inadequate to demonstrate a subtle effect of low amounts of alcohol on fibrosis." I think this is a very cautious interpretation of their data. One could also reasonably conclude that low amounts of alcohol are probably not detrimental to HCV-infected patients and that one drink a day may have more cardiac benefit than it has fibrogenic detriment, as consistent with the NIH consensus statement. I'll drink to that. (See HEPATOLOGY 2004;39:826-834.)

B Still My Liver: Long-Term Benefits of IFN in Chronic Hepatitis B

If you've ever wondered about the long-term outcome of persons with chronic hepatitis B who respond to IFN, the answer is quite favorable. van Zonneveld and coworkers in the Netherlands studied 165



HBsAg-positive patients for a median of 8.8 years after IFN therapy given at a median dose of 30 million units for a median of 16 weeks. After this 4-month course, 33% had lost HBsAg within 12 months of the end of therapy; 13% of these reactivated, so the net sustained loss of HBsAg was 28% (Fig.). Response was best in those who had the highest pretreatment ALT and highest level of inflammatory activity on biopsy, again confirming that those who have the most preexistent immune-mediated liver damage respond the best to therapy. Surprisingly, patients with preexistent cirrhosis had a higher response rate than those without cirrhosis (50% vs. 29%; $P = .02$). While the overall response rate was not dramatic, in those who did respond the long-term outcome was dramatically improved: 52% of responders lost HBsAg compared with 9% of nonresponders ($P < .001$), and 70% lost HBV DNA by PCR compared with 30% of nonresponders ($P < .001$). Further, fewer responders had progression of fibrosis during follow-up ($P = .039$). When survival was adjusted for preexistent cirrhosis, responders with and without cirrhosis had improved sur-

vival, and most strikingly, of the 8 cases of HCC that developed during follow-up, 6 were in nonresponders and 1 was in a responder who relapsed. This decrease in evolution to HCC is even more remarkable considering that the responder group had twice as many cases of cirrhosis and thus was at greater risk for HCC. Thus, while better and/or additional medications are clearly needed to increase the proportion of persons with chronic hepatitis B who have sustained responses, the approximate 30% who do respond experience significant loss rates of HBsAg, HBeAg, and HBV DNA, and significantly decreased inflammation, fibrosis progression, and HCC risk that combine to significantly improve survival compared with nonresponders. (See HEPATOLOGY 2004;39:804–810.)

SARS-Associated Hepatitis: Very SARSprising!

SARS has captured the headlines for its lethal respiratory consequences, but other organ disease has not been prominent except as a terminal event. Now Chau et al. report three cases of hepatitis in which SARS-associated coronavirus (SARS CoV) has been found in liver tissue by PCR. SARS CoV is an enveloped, single-stranded, positive-sense RNA virus that replicates in the cytoplasm of infected cells, features common to HCV. More than 8,000 persons have developed the SARS syndrome, predominantly in Asia, and the fatality rate is about 10%, increasing in the elderly. I was not aware that 60% of SARS patients have elevated ALT levels; these have been difficult to interpret because of multiple potentially hepatotoxic agents, including antibiotics, ribavirin, and protease inhibitors, that are used to treat these patients. In the 3 patients reported, ALT peaked at 493, 475, and 941 IU/L. Bilirubin and coagulation factors were normal, and there was no lactic acidosis. Liver biopsy showed ballooning, acidophilic bodies, and mild to moderate lobular lymphocytic infiltration. Importantly, there was no granuloma, eosinophilic infiltration, cholestasis, fibrosis, fibrin deposition, giant mitochondria, or microvesicular or macrovesicular steatosis. The most striking finding was a large number of cells in mitosis, readily seen by both light and electron microscopy. The hyperproliferative state was also shown by immunohistochemical stains for the proliferative antigen Ki-67; 0.5% to 11.4% of nuclei were positive for Ki-67. The conspicuous mitosis seemed out of proportion to the extent of liver damage. All 3 patients had evidence for apoptosis, the presumed mechanism of liver damage. RT-PCR for SARS-CoV was positive in the liver but not in the serum, so the hepatic localization was not a matter of serum contamination. Viral particles were not seen in the liver. All 3 patients recovered completely from their liver disease. The SARS CoV is quite distinct from other coronaviruses, but a group 2 coronavirus has been shown to cause liver disease in mice and to induce similar pathologic changes. I normally would not highlight Case Reports, but SARS is a hot topic, and confirmed SARS-associated acute hepatitis is a novel observation I find intriguing. Now that it has been reported, can other cases be SARS behind? (See HEPATOLOGY 2004;39:302–310.)

Compartment Living: What's a Nice Virus Like You Doing in a Place Like This?

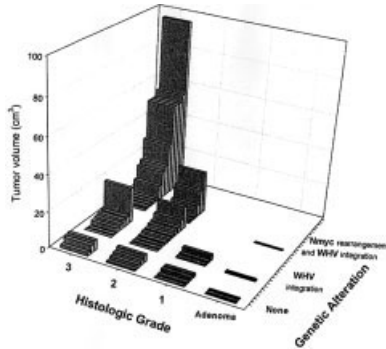
The nearly 100% infection of HCV-naïve liver in the HCV-transplant setting has always served as *prima facie* evidence for extrahepatic sites of HCV replication. Many studies have detected HCV RNA in peripheral blood mononuclear cells (PBMC), and some have demonstrated low-level replication based on the finding of negative-strand replicative intermediates. Ducoulombier and coworkers carry this a step farther by examining the HCV quasispecies in various PBMC compartments. The findings are quite interesting, not because the researchers found HCV in several PBMC compartments, but because they found differences in the HCV quasispecies composition between the plasma and PBMC and among different PBMC compartments. HCV RNA was found in 11 out of 11 CD 19+ B cells, in 10 out of 11 CD 14+ monocytes, and in 4 out of 8 CD8+ T effector cells, but in no CD4+ T helper cells. By cloning and sequencing to detect quasispecies variation in HVR1 and determining the ratio of nonsynonymous to synonymous (d_N/d_S) sequence changes and by phylogenetic tree analysis, they found significantly different sequences between HCV in plasma and at least one cellular compartment in all cases; B cells and monocytes were the main PBMC subsets involved in HCV compartmentalization, and the variants harbored by B cells and monocytes were always different from each other. By measuring IgG-bound versus free HCV, they showed that compartmentalization to cells was not simply binding of HCV complexes through Fc receptors. Overall, the authors conclude, “differences in d_N/d_S ratios between compartments, together with the statistically significant compartmentalization of HCV variants, may point to autonomous HCV replication in blood B lymphocytes and/or monocytes.” Evidence from this and other studies suggests that these cell-compartmentalized variants replicate slowly and contribute little to the plasma viral load. These data suggest that distinct HCV variants within PBMC are under less immune pressure and not only might play a role in HCV persistence, but also might represent a quiet harbor of slowly replicating forms biding their time to sail into the next available liver. (See HEPATOLOGY 2004;39:817–825.)

Knockin' Around With HCC

Tired of knockout mice? Then try some knockins, now available at knockoff prices. Wang et al. generated two HBV gene knockin transgenic mouse lines containing the complete genes of HBsAg and HBx, each inserted into the same p21 locus. Between the ages of 15 and 24 months, 53% of male HBsAg heterozygotes and 72% of male HBsAg homozygotes developed HCC; no female mice developed HCC within that time. In the HBx knockins, 62% of males and 44% of females developed HCC. None of the wild-type controls developed HCC. In gene array analysis, expression of the estrogen receptor ER- β was extremely elevated only in the liver tumors of male HBsAg transgenic mice, fitting with the male-female HCC disparity noted above and suggesting that the ER- β -signaling pathway might be involved in the male preponderance observed during the development of HBsAg-related HCC in humans. These

data also suggest that ER- β -specific ligands may be excellent targets for new HCC treatment modalities. This study of knockins is a knockout. (See HEPATOLOGY 2004;39:318–324.)

How Many Proto-Oncogenes Can A Woodchuck Chuck?



The woodchuck has been a wonderful model for the study of viral-induced HCC. Nearly 100% of woodchucks experimentally infected with woodchuck hepatitis virus (WHV) at birth develop HCC. The

study by Jacob et al. examines the frequency of WHV integration and N-myc oncogene rearrangements and relates these to tumor size and the extent of dysplasia in 55 tumor nodules from 13 chronic WHV carriers. The percent integration / N-myc rearrangement increased from 43% / 0% for grade 1 HCC to 80% / 38% for grade 2 HCC, and 79% / 74% for grade 3 HCC. The larger the tumor and the more advanced the grade, the higher the proportion of integrations and oncogene rearrangements (Fig.); 24 out of 26 N-myc-2 rearrangements were associated with WHV integration, but 2 were isolated. In almost all tumors, the abnormal N-myc-2 fragment comigrated with the WHV DNA integrant, further emphasizing their important interaction.

What are we to make of this? It would be logical to think that viral integration precedes oncogene activation, which in turn incites tumor evolution and growth. This sequence may indeed be correct in some cases, but it appears that early, small tumors frequently lack both WHV integration and N-myc rearrangement. Hence, these phenomena may not be the initiators of tumorigenesis, but rather the outcome and then perpetuator of the process. In essence, integration and N-myc oncogene rearrangements may be vitally important, but not a *sine qua non* of tumor formation. However, cells with viral integrations and oncogene activation probably have a selective advantage and then clonally expand so that as the tumor grows and becomes

more dysplastic, the proportion of tumor nodules harboring these facilitative events markedly increases. Can these events in woodchuck HCC be directly translated to human HCC? Yes and no. In humans, HBV DNA integrations are randomly distributed throughout the genome and are less frequently associated with specific growth regulatory genes, and clearly HCC is a much less common outcome in neonatally infected children than in experimentally infected woodchucks. In humans, perhaps only when the random integration hits the right oncogenic site does the opportunity for clonally selective, neoplastic growth occur. In addition, tumorigenesis is not unidimensional, and in a proportion of cases, other mechanisms may prevail in the pathogenesis of both woodchuck and human HCC. (See HEPATOLOGY 2004;39:1008–1016.)

MiniHighlights

- A meta-analysis of 1,013 patients from 3 randomized trials showed that peginterferon- α 2a monotherapy significantly reduced fibrosis compared with conventional IFN- α 2a monotherapy and that this effect was seen in sustained viral responders and not in nonresponders (odds ratio 1.61). Patients with BMI <30kg/m² and high baseline ALT had the greatest probability of histological improvement. This outcome counters previous claims that IFN has long-term benefit even in the absence of viral clearance. (See HEPATOLOGY 2004;39:333–342.)

- Bräu et al. examined whether a 16-week delay in administration of ribavirin in HIV/HCV-coinfected patients receiving conventional IFN had benefit compared with those receiving ribavirin at initiation of therapy. Overall, SVR was low in both groups (11.3% vs. 5.6%) and not significantly different. The low SVR reflects the suboptimal treatment (absence of pegylation), frequent ribavirin dose reductions, the coexistence of HIV, and the high rate of discontinued therapy (51%). Not unexpectedly, patients on ribavirin had more anemia in the first 16 weeks, exaggerated in those receiving AZT. HIV infection improved during therapy for HCV. No severe adverse effects due to HIV-HCV drug interactions were noted. Since therapies have advanced since this study was initiated, its relevance is diminished, and this is always the problem in complex long-term clinical trials in a field that is rapidly evolving. (See HEPATOLOGY 2004;39:989–998.)