Two Distinct Subtypes of Hepatitis B Virus–Related Acute Liver Failure Are Separable by Quantitative Serum Immunoglobulin M anti-Hepatitis B Core Antibody and Hepatitis B Virus DNA Levels

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Hepatitis B virus (HBV)-related acute liver failure (HBV-ALF) may occur after acute HBV infection (AHBV-ALF) or during an exacerbation of chronic HBV infection (CHBV-ALF). Clinical differentiation of the two is often difficult if a previous history of HBV is not available. Quantitative measurements of immunoglobulin M (IgM) anti–hepatitis B core antibody (anti-HBc) titers and of HBV viral loads (VLs) might allow the separation of AHBV-ALF from CHBV-ALF. Of 1,602 patients with ALF, 60 met clinical criteria for AHBV-ALF and 27 for CHBV-ALF. Sera were available on 47 and 23 patients, respectively. A quantitative immunoassay was used to determine IgM anti-HBc levels, and real-time polymerase chain reaction (rtPCR) was used to determine HBV VLs. AHBV-ALFs had much higher IgM anti-HBc titers than CHBV-ALFs (signal-to-noise [S/N] ratio median: 88.5; range, 0-1,120 versus 1.3, 0-750; $P < 0.001$); a cut point for a S/N ratio of 5.0 correctly identified 44 of 46 (96%) AHBV-ALFs and 16 of 23 (70%) CHBV-ALFs; the area under the receiver operator characteristic curve was 0.86 ($P < 0.001$). AHBV-ALF median admission VL was 3.9 (0-8.1) log10 IU/mL versus 5.2 (2.0-8.7) log10 IU/mL for CHBV-ALF ($P < 0.025$). Twenty percent (12 of 60) of the AHBV-ALF group had no hepatitis B surface antigen (HBsAg) detectable on admission to study, wheras no CHBV-ALF patients experienced HBsAg clearance. Rates of transplant-free survival were 33% (20 of 60) for AHBV-ALF versus 11% (3 of 27) for CHBV-ALF ($P = 0.030$). *Conclusions:* AHBV-ALF and CHBV-ALF differ markedly in IgM anti-HBc titers, in HBV VLs, and in prognosis, suggesting that the two forms are, indeed, different entities that might each have a unique pathogenesis. (HEPATOLOGY 2012;55:676-684)

Hepatitis B virus (HBV)-related acute liver failure (ALF) constitutes 1% of those experiencing acute or chronic HBV (AHBV-ALF and CHBV-ALF, respectively).[1,2] Patients who have AHBV-ALF, as well as those with an acute exacerbation (i.e., disease flare) of CHBV-ALF, cannot be distinguished on clinical grounds without historical or histological evidence for chronicity, which may be lacking in acutely ill patients. CHBV-ALF may occur spontaneously or as a result of the effect of immunosuppression on viral replication and immunity.[1,2] We postulated that serological or virological factors might better separate acute

Abbreviations:: AHBV-ALF, acute HBV infection; ALF, acute liver failure; ALT, alanine aminotransferase; ANCOVA, analysis of covariance; anti-HBc, anti–hepatitis B core antibody; AST, aspartate aminotransferase; AUROC, area under the receiver operating characteristic curve; CHBV-ALF, chronic HBV infection; HCC, hepatocellular carcinoma; CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; IgM, immunoglobulin M; HIV, human immunodeficiency virus; INR, international normalized ratio; LLD, lower limit of detection; OR, odds ratio; ROC, receiver operating characteristic; rtPCR, real-time polymerase chain reaction; S/N, signal-to-noise ratio; VL, viral load.

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Received January 19, 2011; accepted September 27, 2011.

This study was funded by a National Institutes of Health grant (DK U-01 58369) for the Acute Liver Failure Study Group provided by the National Institute of Diabetes and Digestive and Kidney Diseases. Additional funding was provided by the Tips Fund of the Northwestern Medical Foundation, the Jeanne Roberts and Rollin and Mary Ella King Funds of the Southwestern Medical Foundation, and T-32 DK007745-12 (to D.D.).

**Members of the Acute Liver Failure Study Group are listed in the Appendix.*

infections from acute exacerbations of chronic disease when they present as ALF, because the immune pathogenesis of each may be somewhat different.

During the natural history of HBV infection, immune response and degree of liver injury, as exemplified by aminotransferase levels, are considered to be roughly inversely proportional to HBV viral loads (VLs), which vary widely from over 1 billion copies in immune-tolerant patients to barely detectable or negative in inactive carriers.[3-5] A strong adaptive immune response results in rapid clearance of hepatitis B surface antigen (HBsAg) and early detection of antibodies to HBsAg in some patients with HBV-ALF.[6] In support of this, low or undetectable HBV VLs or HBsAg can be observed in approximately 20% of such patients.[1,7] By contrast, in CHBV-ALF, accompanied by immunosuppression, the virus may become directly cytopathic, whereas liver injury in chronic infected patients who are not immunosuppressed is still presumed to be immune mediated.[8]

Detection of immunoglobulin M (IgM) anti–hepatitis B core antibody (anti-HBc), measured by enzyme-linked immunosorbent assay (ELISA), is critical in differentiating AHBV-ALF from CHBV-ALF. However, patients with CHBV-ALF sometimes demonstrate IgM anti-HBc positivity.[9,10] Previous semiquantitative assays described the longitudinal changes in IgM anti-HBc, but no studies have provided direct assessments of IgM anti-HBc quantitation in patients with ALF.[11] Use of a semiquantitative IgM anti-HBc ELISA, rather than a single cut-off value, might better distinguish AHBV-ALF from CHBV-ALF. In addition, measurement of VLs across a wide dynamic range has not been studied extensively and might provide a second tool to separate AHBV-ALF from CHBV-ALF.

In the present study, we classified a large group of patients, all of whom met criteria for HBV-related ALF, separating them on historical and clinical grounds into either AHBV-ALF or CHBV-ALF. We then determined whether quantitative measurement of IgM anti-HBc or HBV VLs (or a ratio combining the two), performed in blinded fashion, could help to distinguish between AHBV-ALF and CHBV-ALF.

Patients and Methods

Patients. Between January 1998 and December 2009, 23 sites in the U.S. ALF Study Group enrolled 1,602 patients with ALF comprising all etiologies, to study, in a prospective fashion, their clinical characteristics and outcomes. The definition of ALF included severe acute liver injury without known cirrhosis, with a duration of illness of <26 weeks accompanied by hepatic encephalopathy and coagulopathy (prothrombin time ≥ 15 seconds or international normalized ratio [INR] ≥ 1.5)[12]; 105 patients were screened and 87 patients with HBV-ALF met criteria for enrollment, as outlined in Fig. 1. All patients were either IgM anti-HBc positive or HBsAg positive or both; 12 HBsAg-negative/IgM anti-HBc–positive patients were considered to represent early viral clearance.[7] The clinical distinction between AHBV-ALF and CHBV-ALF was made after careful review of each case report form by one of us (W.M.L.), using specific criteria from the clinical history. Chronic patients either had a known history of having chronic disease (i.e., previous evidence of HBV at least 6 months before admission to study) or, in the setting of immunosuppression or human immunodeficiency virus (HIV) infection, ALF was assumed to represent chronic infection. The AHBV-ALF group was characterized by age <50 plus high-risk behaviors, such as injection drug use, multiple sex partners, or sex with a known HBV carrier; in the absence of chronic disease or high-risk behavior, older age (>50 years) and Asian ethnicity were deemed to indicate chronicity. Fifteen patients could not be characterized using these criteria. The reviewer was unaware of VLs or quantitative IgM anti-HBc levels when the adjudication was made. Standard molecular analyses, using polymerase chain reaction (PCR) followed by standard consensus sequencing, were used for viral genotyping ($n = 71$) and determining the presence of hepatitis Be antigen (HBeAg)-negative mutations ($n = 68$).

Because patients, by definition, were encephalopathic, informed consent was obtained from next of kin before study enrollment. The study was approved by the local institutional review board at each site. Detailed demographics as well as clinical and outcome data of all HBV-related ALF patients were available from the coordinating center (University of Texas Southwestern Medical Center, Dallas, TX). Serum samples were collected serially for up to 7 days after

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DOI 10.1002/hep.24732

Potential conflict of interest: Dr. Lee consults for and received grants from Novartis. He consults for Eli Lilly. He also received grants from Bristol-Myers Squibb, Siemens, Merck, Gilead, and Boehringer-Ingelheim.

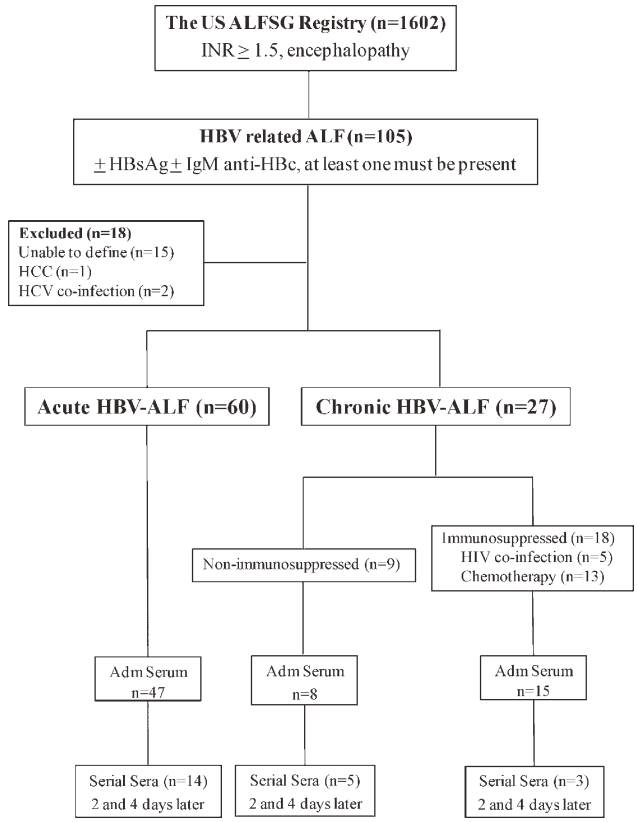

Fig. 1. Study schema. Of the 1,602 ALF patients in the U.S. ALF Study Group, there were 105 HBV-ALF patients identified. Eighteen were excluded: 2 coinfected with hepatitis C virus, 1 determined to have HCC, and 15 who we were unable to define as either acute or chronic. Sixty were identified as AHBV-ALF, of whom 47 had sera collected on admission and 14 had sera collected serially up to day 49. Twenty-seven patients were identified as CHBV-ALF; 9 appeared to show spontaneous exacerbation (nonimmunosuppressed CHBV-ALF), and 18 were considered immunosuppressed; admission and serial sera over 4 days in these groups are also listed.

admission to study and were stored at −80°C before retrieval from the coordinating site for the study. Admission sera used for VL and IgM anti-HBc determinations were considered to be the first available serum samples after enrollment in the study (Fig. 1). Spontaneous survival indicated survival without transplantation, whereas overall survival included all those surviving at 3 weeks after admission to study, regardless of transplantation.

Laboratory Testing.

Measurement of IgM anti-HBc titers.. IgM anti-HBc titers were measured using the ADVIA Centaur IgM anti-HBc assay (Siemens Diagnostics, Tarrytown, NY). Briefly, this assay is an indirect IgM capture immunoassay using a two-step format, including biotinylated antihuman IgM and a solid phase containing streptavidin-coated microparticles. An index value of ≥1 is considered to be reactive, 0.8-0.99 is a "gray zone" that requires retesting, and <0.8 is nonreactive. Each serum sample of 200 μL was run according to assay instructions to determine the index value (i.e., signal-to-noise [S/N] ratio). Sample results beyond the dynamic range of 9.0 (index value range: 0.05-9.0) were serially diluted with a 1:10 dilution using pooled serum that had been tested for HBc IgM antibody and found negative. The final index value was multiplied by the appropriate dilution factor to give a final "calculated" index result.

Quantification of HBV VL by real-time PCR.. Sera collected in serial fashion on days 1 through 4 after admission to study were quantified using an established real-time PCR (rtPCR) protocol.[13] Each serum sample was run in triplicate, and the median value was selected. Viral DNA extracted from serum was amplified and quantified in a 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA). The dynamic range of the assay was 25 to 2×10^7 IU/mL.

Statistical Analyses. Statistical analyses were performed using IBM SPSS Statistics 19.0 (SPSS, Inc., Chicago, IL), SAS 9.2 (SAS Institute, Inc., Cary, NC), and StatXact V8 (Cytel Inc., Cambridge, MA). The Mann-Whitney U test was used to compare groups (AHBV-ALF versus all CHBV-ALF or only the non-immunosuppressed CHBV-ALF group) on continuous measures, including HBV VL and IgM anti-HBc levels. Chi-squared or Fisher's exact tests (when appropriate) were employed to compare groups on categorical measures. The receiver operator characteristic (ROC) curve and the test for the area under the receiver operator characteristic curve (AUROC) were used to describe the relationship between the true-positive rate and the false-positive rate in the prediction of group membership (AHBV-ALF versus overall CHBV-ALF), using the continuous-measures IgM anti-HBc levels and the ratio of IgM anti-HBc to VL; standard errors for areas were estimated using a nonparametric method and testing the null hypothesis of the true area equal to 0.50. A mixed-model analysis of covariance (ANCOVA) was used to determine the effects of time (i.e., baseline, day 2, and day 4) on log10 VLs for AHBV-ALF versus CHBV-ALF, with subject at each time point treated as a random effect. Covariates considered in this model were patient age, admission IgM anti-HBc levels, and use of nucleoside (tide) analog; covariates remained in the model if $P < 0.15$. Logistic regression analysis was employed to predict AHBV-ALF versus CHBV-ALF using admission log10 VLs, admission IgM anti-HBc titers, admission HBsAg status, patient age, and HBV genotypes (i.e., A, B, and C) as covariates; Hosmer-Lemeshow P values were provided to demonstrate the fit of the model to the

data. AUROC analysis was used to examine the predictions made by the logistic regression analysis in distinguishing between the AHBV-ALF and CHBV-ALF groups. For all statistical tests, a P value <0.05 was considered statistically significant, unless otherwise stated. VL is expressed as the median (range) (log10 IU/mL), unless otherwise stated.

Results

Analysis of the Two Groups: AHBV-ALF and CHBV-ALF. Initially, data on 105 patients were available: We excluded 2 HBV+HCV coinfected patients, 1 patient whose liver biopsy later revealed extensive hepatocellular carcinoma (HCC) and 15 who could not be characterized as to their acute or chronic status (Fig. 1). Comparing these 15 unclassified cases to the remaining 87, race ($P = 0.223$) and gender ($P = 0.576$) were not different; however, the median age (range) for the 15 not included was significantly older than those that remained in the analysis (55.5 [40-69] versus 41 [17-71], respectively; $P = 0.001$). Thus, 60 met criteria for the AHBV-ALF group and 27 for the CHBV-ALF group. No patient in either group was coinfected with hepatitis A, D, or E viruses using standard tests. Liver histology, available for 31 of the overall group, did not show evidence of cirrhosis in any patient.

The 27 patients within the overall CHBV-ALF group included 14 who were known to have CHBV-ALF. Nine experienced spontaneous acute exacerbation (unexplained and without immunosuppression), whereas the remaining 18 experienced immunosuppression-related ALF: 13 had received either chemotherapy for leukemia or lymphoma (n = 9), or corticosteroids for Crohn's disease, asthma, Guillain-Barre syndrome, and unknown (1 each); 5 had concomitant HIV infection. Of interest, 6 of the 18 immunosuppressed patients were unaware of their diagnosis of CHBV-ALF at the time of presentation. The remaining 9 CHBV-ALF patients with apparent acute or chronic disease included 5 known to have CHBV-ALF. Among the 60 that were categorized as having true CHBV-ALF, 23 had a history of injection drug use (only) and 19 had a history of high-risk sexual behavior or sex with a known HBV individual; in 5, both risk factors were positive.

In general, clinical and laboratory features, such as length of illness and INR levels, aspartate aminotransferase (AST) levels, bilirubin, and creatinine, did not differ between the 60 patients considered to have AHBV-ALF and the 27 in the overall CHBV-ALF group (Table 1). However, alanine aminotransferase (ALT) levels and albumin levels were higher in the AHBV-ALF patients than the CHBV-ALF patients. There were few apparent differences in virologic or host features found between the two chronic subgroups (9 nonimmunosuppressed versus 18 patients with immunosuppression), although viral loads at admission were lower in the nonimmunosuppressed group. For the remaining statistical analyses, we combined the CHBV-ALF subgroups, except where noted.

Demographics and HBV Genotypes. Certain important demographic and virologic characteristics differed between the two main groups: AHBV-ALF patients were younger (median, 36 years; range, 17-64) than patients with CHBV-ALF (median, 53; range, 33-71; $P < 0.001$; Table 1). The AHBV-ALF group was comprised mostly of Caucasians (31 of 60; 52%) and African Americans (22 of 60; 37%), whereas Asians accounted for only 2 of 60 (3%) of AHBV-ALFs. The AHBV-ALF group included primarily genotypes A (24 of 47; 51%) and D (14 of 47; 30%), with only 4 and 2 patients, respectively, classified as genotype B or C.

By contrast, the CHBV-ALF group included a much larger number of Asians (14 of 27; 52%) and, as expected, mainly genotypes B (11 of 24; 46%) and A (7 of 24; 29%). Mutations in the core promoter region alone did not differ between the two groups, AHBV-ALFs (15 of 48; 31%) versus CHBV-ALFs (5 of 20; 25%) ($P = 0.606$), whereas precore mutations were significantly more common in CHBV-ALFs (10 of 20; 50%) versus AHBV-ALFs (10 of 47; 21%) ($P = 0.019$).

Admission Titers and Cut-off Value of IgM Anti-HBc in AHBV-ALFs Versus CHBV-ALFs. Ninety-six percent (44 of 46) of the AHBV-ALF patients had positive IgM anti-HBc tests (index value, ≥ 1.0), whereas 15 of 23 (65%) of those deemed to have CHBV-ALF tested positive for IgM anti-HBc. Admission IgM anti-HBc index values in AHBV-ALFs (n = 46; median, 88.5; range, 0-1,120) were significantly higher than those of the CHBV-ALF group (n = 23; median, 1.3; range, 0-750) ($P < 0.001$) (Fig. 2). Among patients with CHBV-ALF, 70% (16 of 23) had index values <5. By contrast, 44 of 46 (96%) of AHBV-ALFs demonstrated IgM anti-HBc index values ≥ 5. Based on these data, the proposed cut-off value of IgM anti-HBc to best differentiate AHBV-ALF from CHBV-ALF was 5.0. Using this cutoff, the percent correct for the overall group was 87 with the AUROC of 0.86 ($P < 0.001$) (curve not shown).

Admission and Serial HBV VLs in AHBV-ALF Versus CHBV-ALF. Patients with AHBV-ALF demonstrated lower admission log10 VLs (n = 51;

Table 1. Demographic and Baseline Characteristics of the Study Patients*

Characteristics	AHBV-ALF (n = 60)	CHBV-ALF (n = 27)	*P* Value†	CHBV-ALF Nonimmunosuppressed (n = 9)	*P* Value‡
Age (years)	36 (17-64)	53 (33-71)	<0.001	60 (46-69)	<0.001
Race					
African American	37 (22/60)	22 (6/27)	<0.001	0	<0.001
Asian	3 (2/60)	52 (14/27)		67 (6/9)	
Caucasian	52 (31/60)	22 (6/27)		22 (2/9)	
Other	8 (5/60)	4 (1/27)		11 (1/9)	
Female sex	47 (28/60)	37 (10/27)	0.402	22 (2/9)	0.281
ALT (IU/L)	1,954 (87-11,100)	1,008 (94-7,856)	0.018	826 (117-7,774)	0.069
AST (IU/L)	1,024 (66-9,901)	719 (78-8,615)	0.248	530 (98-7,420)	0.250
Bilirubin (mg/dL)	18.4 (3.6-62.4)	20.2 (5.7-36.0)	0.319	21.4 (7.5-30.4)	0.392
Albumin (g/dL)	2.8 (1.7-4.0)	2.5 (1.7-3.3)	0.006	2.4 (2.0-3.3)	0.056
INR	2.9 (1.2-20.1)	3.2 (0.9-10.3)	0.456	2.7 (0.9-9.4)	0.679
Hepatic coma grade					
I and II	48 (29/60)	41 (11/27)	0.511	33 (3/9)	0.489
III and IV	52 (31/60)	59 (16/27)		67 (6/9)	
Creatinine (mg/dL)	1.1 (0.4-8.6)	1.1 (0.6-10.5)	0.508	1.8 (0.6-6.6)	0.269
Days from jaundice to onset of hepatic coma	5 (0-46)	9.5 (0-28)	0.277	13 (1-28)	0.202
HBsAg negativity	20 (12/60)	0 (0/27)	0.015	0 (0/9)	0.342
HBeAg positivity	26 (15/57)	27 (7/26)	0.954	11 (1/9)	0.436
Anti-HBs positivity	28 (17/60)	15 (4/27)	0.173	11 (1/9)	0.428
HBV genotype					
A	51 (24/47)	29 (7/24)	0.003	13 (1/8)	<0.001
B	9 (4/47)	46 (11/24)		88 (7/8)	
C	4 (2/47)	8 (2/24)		0	
D	30 (14/47)	17 (4/24)		0	
Other	6 (3/47)	0		0	
HBV basal core promoter mutations (A1762T/G1764A)	31 (15/48)	25 (5/20)	0.606	29 (2/7)	>0.999
HBV precore mutation (G1896A)	21 (10/47)	50 (10/20)	0.019	86 (6/7)	0.002
HBV precore ± basal core promoter mutations	40 (19/47)	60 (12/20)	0.141	86 (6/7)	0.041
HBV VL, $\log_{10}$ (IU/mL)	3.9 (0.0-8.1)	5.2 (2.0-8.7)	0.025	3.8 (2.5-8.7)	0.982
IgM anti-HBc (index value)	88.5 (0-1,120)	1.3 (0-750)	<0.001	1.9 (0-28.9)	<0.001
N-acetylcysteine treatment	51 (30/59)	15 (4/27)	0.002	44 (4/9)	>0.999
Acetaminophen use mentioned	80 (28/35)	60 (6/10)	0.228	67 (2/3)	0.519
Alcohol use	43 (25/58)	31 (8/26)	0.285	33 (3/9)	0.724
Nucleoside (tide) analogs use	53 (31/59)	78 (21/27)	0.026	89 (8/9)	0.068
Spontaneous survival	33 (20/60)	11 (3/27)	0.030	11 (1/9)	0.258
Overall 3-week survival	72 (43/60)	44 (12/27)	0.015	44 (4/9)	0.132

*Continuous data are shown as median (range) and categorical data are % (numerator/denominator).
†Comparison between AHBV-ALF versus overall CHBV-ALF.
‡Comparison between AHBV-ALF versus CHBV-ALF without immunosuppression (CHBV-ALF).

median, 3.9; range, 0-8.1 log10 IU/mL) than those in the overall CHBV-ALF group (n = 24; median, 5.2; range, 2.0-8.7 log10 IU/mL; Fig. 3); the difference in median levels between groups was between 1 and 2 logs. Of note, there was considerable overlap of admission VLs between AHBV-ALFs and CHBV-ALFs. There were 4 patients in the AHBV-ALF category who had undetectable admission VLs by our assay (lower limit of detection [LLD]: 25 IU/mL), compared to none with CHBV-ALF. A difference was observed in the viral loads for the two CHBV-ALF subgroups, in that the nonimmunosuppressed group median VL was similar to that of the acute group and less than that of the immunosuppressed group, as might be expected.

Overall, high IgM anti-HBc and low VLs characterized the AHBV-ALF group, whereas the opposite was true of the CHBV-ALF subjects (Figs. 2 and 3).

Changes in VLs Over Time. Mean log10 VLs declined significantly for both groups at three time points over 4 days ($P < 0.001$; Fig. 4). With relatively low initial levels, the VLs in AHBV-ALFs continued to decline and were consistently lower at all time points than the mean CHBV-ALF log values ($P < 0.001$). The interaction between the two groups over time was nonsignificant ($P = 0.360$), and the only covariate remaining in the ANCOVA model ($P < 0.150$) was admission IgM anti-HBc levels ($P = 0.137$).

Ratio of IgM Anti-HBc to HBV VL. The ratio of IgM anti-HBc to HBV VL, calculated using admission

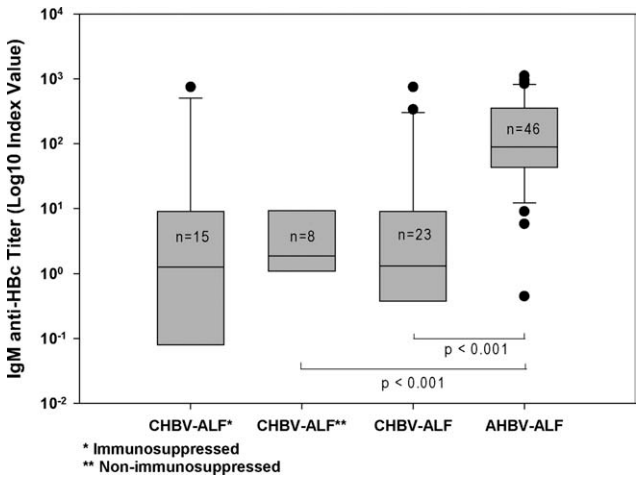

Fig. 2. IgM anti-HBc levels for the various groups. Admission IgM anti-HBc levels were much higher in AHBV-ALFs than in the overall CHBV-ALF group. Median IgM anti-HBc index value (S/N) on admission to hospital in the 46 AHBV-ALFs was 88.5 (range, 0-1,120), significantly higher than that of the 23 overall CHBV-ALFs (1.30 [0-750]; $P < 0.001$) or the 8 nonimmunosuppressed CHBV-ALFs [**] (1.9 [0-28.9]; $P < 0.001$]. The median (range) value for the 15 immunosuppressed CHBV-ALFs [*] was 1.27 (0-750). There was no difference observed between the two CHBV-ALF subgroups.

values for each patient, was significantly higher in AHBV-ALF (median, 9.2×10^{-3}; range, 0-1.1) than in CHBV-ALF (median, 1.0×10^{-5}; range, 0-2.0×10^{-2}; $P < 0.001$; AUROC, 0.86; $P > 0.001$). Logistic regression analysis, considering VLs, IgM anti-HBc titers, age, HBV genotypes (i.e., A, B, and D), and HBsAg status upon admission, was employed to determine independent predictors of AHBV-ALF versus CHBV-ALF. Admission log10 VLs ($P = 0.022$; odds ratio [OR], 0.569; 95% confidence interval [CI]: 0.352-0.921), IgM anti-HBc ($P = 0.005$; OR, 1.006; 95% CI: 1.002-1.010), and age ($P < 0.001$; OR, 0.855; 95% CI: 0.788-0.929) were independent determinants that distinguished the groups (Hosmer-Lemeshow, $P = 0.812$; AUROC = 93%; $P < 0.001$). There were 3 "outlier" patients in the CHBV-ALF group who exhibited high IgM anti-HBc titers of 248, 337, or 750 index values with correspondingly high HBV VLs on admission (1.21×10^3, 20.05×10^6, and 9.70×10^6). However, the ratios of IgM anti-HBc to HBV VLs were 2.04×10^{-2}, 1.68×10^{-5}, and 7.73×10^{-5}, respectively, suggesting that they may, indeed, belong to the CHBV-ALF group. Two were receiving an immunosuppressive agent and the 3rd had HIV coinfection.

HBsAg Status and Outcomes. All CHBV-ALF patients were HBsAg positive, whereas 20% (12 of 60) of AHBV-ALF had undetectable HBsAg on admission ($P = 0.015$). Median IgM anti-HBc index values and log10 VL for the 12 HBsAg-negative patients were 125 (range, 17.2-840) and 3.55 (range, 0-8.09) log10 IU/mL, respectively. Spontaneous survival was significantly higher for AHBV-ALF patients (33%; 20 of 60) than for those with CHBV-ALF (11%; 3 of 27) ($P = 0.030$). Overall survival was higher for AHBV-ALF patients (72%; 43 of 60) than for those with CHBV-ALF (44%; 12 of 27) ($P = 0.015$). Admission HBeAg,

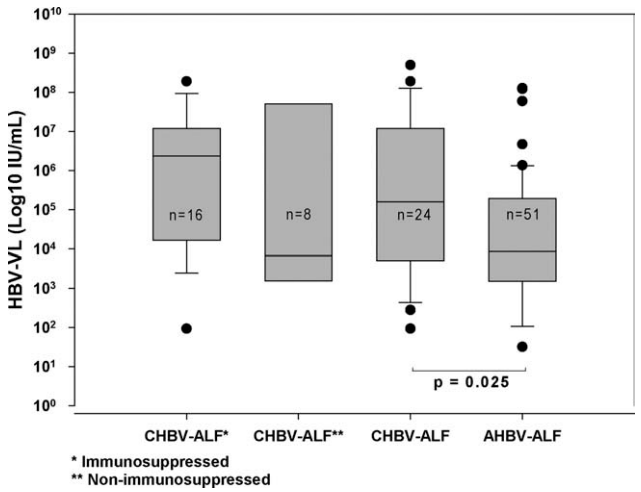

Fig. 3. HBV VLs for the various groups. Median admission VL in 51 patients with AHBV-ALF was 3.9 (0-8.1) log10 IU/mL, significantly lower than observed for the 24 patients in the overall CHBV-ALF group (5.2 [2.0-8.7] log10 IU/mL; $P = 0.025$), but not for the 8 nonimmunosuppressed CHBV-ALF patients (3.8 [2.5-8.7] log10 IU/mL; $P = 0.982$). The median (range) for the 16 in the immunosuppressed subgroup was 6.28 (1.97-8.28) log10 IU/mL. There were no significant differences in admission VLs between the two CHBV-ALF subgroups; horizontal line in each bar graph represents median VL.

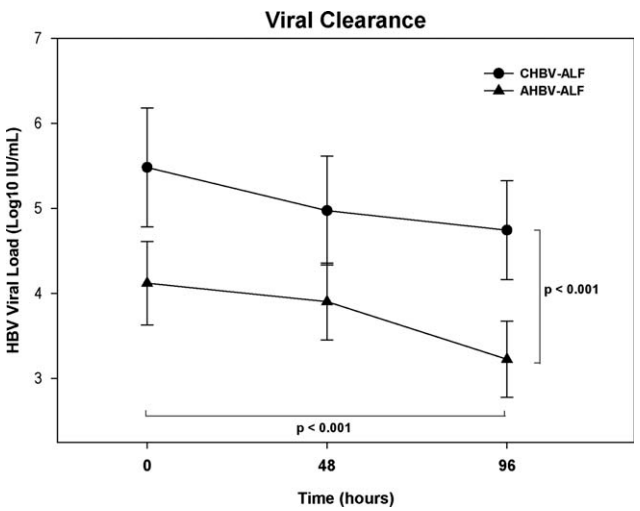

Fig. 4. ANCOVA for VLs measured over time for AHBV-ALF and the overall CHBV-ALF groups, adjusting for baseline IgM anti-HBc levels. The decrease in VLs was significant for each of the two groups ($P < 0.001$). VLs in AHBV-ALFs were consistently lower than in CHBV-ALFs at all time points ($P < 0.001$). Admission IgM anti-HBc level was the only covariate that remained significant in the ANCOVA model ($P = 0.137$). Error bars are 95% CIs.

Anti-HBs positivity, and coma grade were comparable between the two groups (Table 1). Follow-up (beyond 3 weeks) was available on 15 of 22 with AHBV-ALF who had survived without grafting. This group would be expected to clear HBsAg if they represented "true acute" infections: 4 had died after 3 weeks, including 2 who had undergone liver grafting, and 4 had cleared their infection, as indicated by negative HBsAg at 3 weeks to 18 months after infection (no earlier visits for this patient). In 7, further follow-up failed to include information on HBsAg clearance, and 7 others were lost to follow-up.

Discussion

This study sought to describe, in more detail, the clinical and immunological features of patients with AHBV-ALF or CHBV-ALF in relation to their serological features and molecular biology, because distinguishing between the two forms might well have clinical and pathogenetic significance. There were at least two distinct groups within the overall HBV-ALF cohort, based on history obtained and certain key clinical and histological features: One with newly acquired acute HBV infection leading to ALF (illness <6 months, usually <2-4 weeks) and one in which ALF had occurred in the setting of definite or presumed chronic disease. The source of confusion has been that the two forms resemble each other remarkably in clinical and biochemical features, such as apparent rapid onset of severe disease, advanced grades of encephalopathy, high aminotransferases, and prolonged INRs, and thus cannot be distinguished readily without historical information of chronicity or, by contrast, of recent HBV exposure, both of which are often lacking. Although we had made initial assessments on overall gestalt, we revisited the data on each conditional random field using the algorithm described above, including, as primary data, confirming CHBV-ALF with either previous knowledge of CHBV-ALF, whether the patient was receiving chemotherapy, or had HIV coinfection. Next, we considered evidence of high-risk behavior, age, and ethnicity to complete the picture. This was not a 100% accurate profiling procedure, because it was performed blindly with only clinical historical data, with possible proof being that, by this technique, 2 with negative IgM anti-HBc were classified as being acute. However, at least 85% of our assessments are likely to be accurate, based on these criteria. Among those misclassified might be, for example, a young patient with high-risk behavior who might have CHBV-ALF. We were forced to exclude 15 patients from the original cohort because features delineating acute from chronic could not be found. It is well known, however, that ALF patient histories are often limited by the presence of encephalopathy on arrival at the referring hospital.

The acute form of HBV-ALF accounted for two thirds of the overall group; CHBV-ALF comprised the rest, 2 of 3 of whom were considered immunosuppressed. Other acute-on-chronic patients in the cohort might have been excluded because they lacked adequate history of chronicity. CHBV-ALF subjects receiving immunosuppressive agents or coinfected with HIV were considered together in this analysis. The immunosuppressed group differed in only minor respects from the remainder of the CHBV-ALF group. However, one differentiating feature was VL, which was lower in the nonimmunosuppressed group.

HBV VL, IgM anti-HBc titer, or the ratio of the two (if both values are available) effectively distinguished the two forms of ALF resulting from HBV, particularly the quantitation of IgM anti-HBc levels, with an AUROC of 86%. In practice, the presence of IgM anti-HBc positivity is associated with acute infection and is necessary, but not sufficient, to diagnose AHBV, because IgM anti-HBc is also observed, in some patients, with exacerbation of chronic infection.[10,14] Higher IgM anti-HBc titers have been suggested to be associated with a highly active host immune response. Quantitative IgM anti-HBc testing more accurately distinguishes between acute and nonacute cases. Fink et al. showed that a strong immunologic response promotes B-cell differentiation into IgM-producing plasmablasts and high titers of IgM antibody, whereas moderate or weak stimulation drives differentiation into memory or plasma cells that mostly produce the immunoglobin G isotype.[15] Moreover, clinical studies have consistently suggested that higher titers of IgM anti-HBc are observed in new AHBV than in CHBV.[16] The cut-off index value for IgM anti-HBc of 5.0 in our study effectively differentiated AHBV-ALF from CHBV-ALF with a positive predictive value of 86% and a negative predictive value of 89%. Though 35% of CHBV-ALF patients demonstrated levels of IgM anti-HBc between 1.0 and 5.0 using the ADVIA assay (Siemens Diagnostics), 70% were below the index value of 5.0. Given our current knowledge, we suggest that determining the IgM anti-HBc level with a quantitative assay or at least a higher cut-off value than 1.0 is a readily available single test with excellent predictive capacity.

Earlier studies describing rapid viral clearance with undetectable HBsAg and VLs in HBV-related ALF were based on insensitive tests, such as immunoelectrophoresis, radioimmunoassay, and, for VLs, dot blot

hybridization, with an LLD of approximately 100,000 IU/mL.[5,7] We could not find recent studies that compared VLs in either acute or fulminant hepatitis B using current highly sensitive assays. Thus, we did not have available known ranges for VL during AHBV or CHBV leading to liver failure. Using an rt-PCR assay with an LLD of 25 IU/mL, nearly all (92%; 47 of 51) of our AHBV-ALF patients had a detectable HBV VL on admission, although many had remarkably low values. AHBV-ALF subjects had significantly lower VLs than CHBV-ALF subjects, although there was some overlap between groups. The reasons for this overlap included possible inaccuracy of the initial clinical assessment that categorized the patients and the overall heterogeneity in timing of admission to study.

Lower VLs in AHBV-ALF, like higher IgM anti-HBc levels, suggest a more robust immune response. Though the CHBV-ALF group demonstrated higher admission VLs (approximately 6 log10 IU/mL, compared to 3-4 log10 IU/mL), nearly all values declined during the study (Fig. 4). The ratio of IgM anti-HBc to VL had a remarkably high AUROC of 0.86 ($P < 0.001$), but was no better than IgM anti-HBc alone (AUROC = 86%; $P < 0.001$).

Spontaneous survival differed between the two groups: 11% in the CHBV-ALF group vs. 33% in the AHBV-ALF patients, which might be the result of differences in survival with older age. Overall survival was similar between the two groups, largely because of the increased numbers of CHBV-ALF patients receiving a liver allograft.

A third feature of AHBV-ALF is early HBsAg clearance, observed in 20% (12 of 60) of patients with AHBV-ALF and in none with CHBV-ALF.[7] Those who cleared HBsAg demonstrated higher median IgM anti-HBc titers and lower HBV VLs than their peers. The combination of lower VL, high IgM anti-HBc titers, and low or undetectable HBsAg on admission in AHBV-ALF suggests that a more robust host immune response occurs in true acute patients than is observed in CHBV presenting as ALF. Based on these data, it may be useful for clinicians to differentiate AHBV-ALF and CHBV-ALF on clinical and serological grounds, either by use of the IgM anti-HBc titers, VLs or, if available, the ratio of the two. As a single value, IgM anti-HBc appears to be robust using the 5.0 cutoff value. Of interest, the immunosuppressed chronic group were mixed in ethnicity, whereas virtually all remaining acute-on-chronic nonimmunosuppressed patients were of Asian heritage, B genotype (7 of 8), and demonstrated precore (6 of 7) and/or basal core promoter mutations.

As expected, the algorithm we used resulted in an increased number of Asian patients in the CHBV-ALF group, and nearly 50% of CHBV-ALF patients had genotype B. Studies from Asia suggest that genotype B is associated with more frequent acute exacerbations and a higher risk of hepatic decompensation and mortality, compared to genotype C, whereas genotype C is associated with more liver cirrhosis and HCC.[17,18] Among Asian patients with presumed AHBV, more genotype B patients developed a fulminant course; this was confirmed in our non-immunosuppresed CHB-ALF patients, with 7 of 8 being genotype B.[19]

Precore (G1896A) and core promoter (A1762T/G1764A) mutations have been considered drivers of severe disease, although these mutations are also found in chronic or asymptomatic HBV infection.[20] A low prevalence of these mutations was detected in two previous series of HBV-related ALF.[21] However, once the groups were separated in our study, it appeared that 60% of CHBV-ALFs (and all but 1 of the nonimmunosuppressed CHBV-ALFs) presented with either precore, core promoter mutations, or both, as compared to 40% of AHBV-ALFs—this was not statistically significant. These findings may point to the different immunopathogenesis in CHBV-ALF, compared to the truly acute cases. Though these data were obtained on consecutive U.S. patients, the patient characteristics observed here are likely unique to the United States and not readily extrapolated to other populations.

Our study had several limitations. Histories are often limited in rapidly evolving severe illnesses, such as ALF, where altered mentation is a criterion for study entry. The case-selection process likely had inaccuracies as noted, because the distinction between AHBV-ALF and CHBV-ALF was based solely on patient history and demographics. Finally, the ADVIA Centaur IgM Anti-HBc assay used is considered to be semiquantitative and was adapted for the quantitation performed here. Nevertheless, the wide differences observed in IgM anti-HBc between the two groups are clear, because the majority of CHBV-ALF patients demonstrated low IgM anti-HBc titers that were within the first dynamic range (no dilution needed).

In conclusion, AHBV-ALF can be separated from CHBV-ALF on clinical grounds when valid historical data are available, but the two may be more readily distinguished by quantitative IgM anti-HBc, VLs, and/or IgM anti-HBc/VL ratios. Additional indicators, such as HBsAg negativity, younger age, non-Asian, and genotype non-B, provide indirect evidence of AHBV-ALF. By contrast, a low or undetectable IgM anti-HBc level, with elevated HBV DNA VL to >5

log10 IU/mL, in a patient with what appeared to be an acute illness, suggest that this patient probably actually suffered from CHBV-ALF. Overall, HBV-related ALF patients carry a poor prognosis, although those with new acute infection appear to fare somewhat better than those with chronic disease. Differentiation between the two subtypes within HBV-ALF may be helpful in determining more appropriate therapeutic strategies.

Acknowledgments: The authors gratefully acknowledge the support provided by the members of The Acute Liver Failure Study Group 1998-2010. The authors further acknowledge all the coordinators from the study sites, as well as the patients and their families who participated in this study.

Appendix

Members and institutions participating in the Acute Liver Failure Study Group 1998-2009 are as follows: W.M. Lee, M.D. (Principal Investigator), George A. Ostapowicz, M.D., Frank V. Schiødt, M.D., Julie Polson, M.D., University of Texas Southwestern, Dallas, TX; Anne M. Larson, M.D., University of Washington, Seattle, WA; Timothy Davern, M.D., University of California, San Francisco, CA (current address: California Pacific Medical Center, San Francisco, CA); Michael Schilsky, M.D., Mount Sinai School of Medicine, New York, NY (current address: Yale University, New Haven, CT); Timothy McCashland, M.D., University of Nebraska, Omaha, NE; J. Eileen Hay, M.B.B.S., Mayo Clinic, Rochester, MN; Natalie Murray, M.D., Baylor University Medical Center, Dallas, TX; A. Obaid S. Shaikh, M.D., University of Pittsburgh, Pittsburgh, PA; Andres Blei, M.D., Northwestern University, Chicago, IL (deceased); Atif Zaman, M.D., University of Oregon, Portland, OR; Steven H.B. Han, M.D., University of California, Los Angeles, CA; Robert Fontana, M.D., University of Michigan, Ann Arbor, MI; Brendan McGuire, M.D., University of Alabama, Birmingham, AL; Raymond T. Chung, M.D., Massachusetts General Hospital, Boston, MA; Alastair Smith, M.B., Ch.B., Duke University Medical Center, Durham, NC; Robert Brown, M.D., Cornell/Columbia University, New York, NY; Jeffrey Crippin, M.D., Washington University, St Louis, MO; Edwin Harrison, Mayo Clinic, Scottsdale, AZ; Adrian Reuben, M.B.B.S., Medical University of South Carolina, Charleston, SC; Santiago Munoz, M.D., Albert Einstein Medical Center, Philadelphia, PA; Rajender Reddy, M.D., University of Pennsylvania, Philadelphia, PA; R. Todd Stravitz, M.D., Virginia Commonwealth University, Richmond, VA; Lorenzo Rossaro, M.D., University of California Davis, Sacramento, CA; Raj Satyanarayana, M.D., Mayo Clinic, Jacksonville, FL; and Tarek Hassanein, M.D., University of California, San Diego, CA. The University of Texas Southwestern Administrative Group included Grace Samuel, Ezmina Lalani, Carla Pezzia, and Corron Sanders, Ph.D., and the Statistics and Data Management Group included Joan S. Reisch, Ph.D., Linda S. Hynan, Ph.D., Janet P. Smith, Joe W. Webster, and Mechelle Murray.

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