**Data Collection**

Patient data was collected using the NIH Biomedical Translational Research Information System (BTRIS). All patients were enrolled in a natural history of liver diseases [NCT00001971] or gastrointestinal diseases [NCT01639924] protocol. Both protocols are approved by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Institutional Review Board (IRB) and each patient gave written informed consent for participation. Demographic and patient characteristic (age, sex, race, history of IVDU, country of birth and origin) and basic laboratory data (aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, albumin, prothrombin time (PT), gamma-glutamyl transferase (GGT), red cell distribution width (RDW), and platelet count (PLT)) were recorded. Virologic data including hepatitis B-e antigen (HBeAg) and hepatitis B-e antibody (HBeAb) status, quantitative serum HBV-DNA, HBV genotype, presence of hepatitis C virus (HCV) antibodies and viremia, and human immunodeficiency virus (HIV) antibody status, and quantitative serum HDV-RNA were also gathered. Only the initial laboratory values were used for analysis. History of IVDU and use of anti-nucleo(s)tide therapy at baseline was recorded. Chronicity of greater than 6 months for HBV/HDV infection was established based on clinical and laboratory findings.

Subjects that were positive for anti-HCV antibodies (HCVAb) but negative for HCV-RNA were considered to have resolved HCV infection and those positive for HCV-RNA were considered to be infected with HCV. Patients were considered infected with HIV if they tested positive for antibodies to HIV.

**Viral Diagnostics**

HBsAg was measured via enzyme immunoassay (EIA) using the Abbott Commander (Abbott Laboratories, Chicago, IL) prior to 10/2008 and enzyme-linked immunosorbent assay (ELISA) using the VITROS® HBsAg assay on the VITROS® Immunodiagnostic System (Ortho Clinical Diagnostics, Raritan, NJ) after 10/2008. HBeAg and HBeAb was assayed by chemiluminescent immunoassay (Mayo Clinic, Rochester, MN) prior to 12/2011 and VITROS® HBeAg and HBeAb assays on the VITROS® Immunodiagnostic System (Ortho Clinical Diagnostics, Raritan, NJ) after 12/2011. HBV quantification was done via PCR by the COBAS® Monitor (Roche Molecular Systems, Blanchburg, NJ) prior to 2011 (quantitative range: 29-110,000,000 IU/mL) and COBAS® AmpliPrep / COBAS® Taqman (Roche Molecular Systems, Blanchburg, NJ) after 2011 (quantitative range: 20-170,000,000 IU/mL).

HDAb was assayed using the combined anti-HDV IgM and IgG ELISA (Mayo Clinic, Rochester, MN). Qualitative detection and quantification of HDV-RNA was performed via real-time PCR (RT-PCR) (ARUP Laboratories, Salt Lake City, UT). The quantitative range of this assay is 2.1-6.8 log IU/mL (120 - 5,800,000 IU/mL) with a lower limit of detection of 1.8 log IU/mL (62 IU/mL). RNA was extracted in duplicate from cryopreserved serum samples using Qiagen Viral RNA Mini kits (Qiagen, Valencia, CA) according to manufacturer’s instructions. The extracted RNA was reverse transcribed in parallel with dilutions of a quantified, full length Delta RNA transcript (to serve as a standard curve) which was calibrated to the WHO 1st International Standard (Paul-Erlich-Institut, Langen, Germany, using random primers and Superscript III RT (Life Technolgies, Grand Island, NY) according to manufacturer’s instructions. Real-time PCR was performed on the resulting cDNA using Taqman Universal Master Mix (Life Technologies) and a duplex of HDV-specific primerprobe sets: primer HDV-879 (GGT GGA GAT GCC ATG CCG), primer HDV-961 (CAG TGA ATA AAG CGG GTT TCC A), FAM-BHQ-labeled probe HDV-899 (CGT CTC GCG TCC TTG TTT CCT CTT CGG G), primer truncYamaFor (GCT ACT CTT CTT TCC CTT CTC TCG TC); primer newYamaRev (CCG ACA AGG AGA GGC AGG A), and VIC-TAMRA-labeled probe YamaP (TCT TGT TCT CGA GGG CCT TCC TTC G). The duplex of primer/probe sets were employed to account for a) potential polymorphisms in the primer/probe binding sites from different patient strains, and b) nucleotide changes that may arise during treatment.

**Cirrhosis Classification**

Patients were classified as cirrhotic based on the FIB-4(1) and APRI(2) scores which were calculated using the following equations: FIB-4 = (age (years)×AST(IU/L))/[PLT(109/L)×ALT1/2 (IU/L).(1) APRI = (AST (IU/L)/ULN of AST (IU/L))/PLT(109/L)×100.(2) A FIB-4 > 3.25 and an APRI > 2 was used to define cirrhosis. Although the FIB-4 and the APRI scores have been tested in chronic HDV and do not perform as well as in HBV mono-infection, they can still provide some clinical utility in the identification of cirrhosis.(3, 4)

**Statistical Analysis**

All statistical analysis was performed using SAS 9.4 (Statistical Analysis Software; SAS Institute, Cary, NC). The data were stratified based on HDAb testing. Baseline patient characteristics were described using frequencies for categorical variables and means (depending on distribution) for continuous variables. Univariate analysis was conducted using Wilcoxon rank-sum test for categorical variables and one-way ANOVA for continuous variables. To ensure significant difference between the means with the application of the one-way ANOVA, the difference was modeled using Tukey’s studentized range procedure. Multivariate analysis using Fisher’s scoring technique was performed to identify risk factors among patients who were HBsAg positive and tested for HDVAb. A 2-sided *P* value of less than 0.05 was considered statistically significant.

References

1. Sterling RK, Lissen E, Clumeck N, et al. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology 2006;43:1317-25.

2. Wai CT, Greenson JK, Fontana RJ, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology 2003;38:518-26.

3. Takyar V, Surana P, Kleiner DE, et al. Noninvasive markers for staging fibrosis in chronic delta hepatitis. Aliment Pharmacol Ther 2017;45:127-138.

4. Da BL, Surana P, Takyar V, et al. Vibration-controlled transient elastography for the detection of cirrhosis in chronic hepatitis D infection. J Viral Hepat 2019.