Implementation of Occult Hepatitis Screening in the Spanish Cohort of HIV-infected Pediatric Patients

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Abstract: Regular screening methods may miss the diagnosis of occult hepatitis B infection and seronegative hepatitis C virus infection in immunocompromised patients. A cross-sectional study within a Spanish cohort of HIV-infected children yielded 6 of 254 (2.4%) possible occult hepatitis B infection cases and 2 of 254 (0.8%) seronegative hepatitis C virus–infected patients. Implementation of occult hepatitis screening in the routine care of these children may be warranted.

Key Words: hepatitis B core antigens, hepatitis C infection, hepatitis B vaccines, HIV, pediatrics

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Just as the burden of non-AIDS morbidity and mortality has changed in the era of highly active antiretroviral therapy, liver disease has emerged as one of the most common non-AIDS–related causes of death among HIV-infected adults.[1] Occult hepatitis B virus (HBV) infection (OBI)[2] and seronegative or serosilent hepatitis C virus (HCV) infection[3] may not be detected by current screening methods but may pose a risk for later hepatic failure when additional immunosuppression or for a highly active antiretroviral therapy switch for immunovirological failure occurs.

A large body of information has been produced in recent years regarding occult hepatitis in adults, but data in HIV-infected children are lacking. This study was designed to investigate the value of including OBI and HCV-seronegative infection markers in our routine hospital screening protocols for HIV-infected pediatric patients by assessing the prevalence of these entities in a large cohort of vertically HIV-infected Spanish children.

METHODS

The Spanish Cohort of HIV-infected Children (CoRISpe) is a national registry of HIV-infected Spanish patients aged 18 years or younger, and currently includes 838 patients in 75 centers. This report represents a cross-sectional study of all vertically HIV-infected children and adolescents from 14 hospitals in CoRISpe during 2011.

At assessment, CD4+ T-lymphocyte absolute count and HIV-1 viral load (VL) were analyzed. HIV-VL was determined by real-time polymerase chain reaction (PCR) technology: 2 centers used the NucliSens EasyMag system (BioMérieux, Marcy L'Etoile, France) for extraction and NucliSens EasyQ system (BioMérieux) for detection and quantification (lower limit of detection, 25 copies/mL), and the remaining centers used the Ampliprep COBAS Taqman system (Roche Diagnostics GmbH, Mannheim, Germany; lower limit of detection, 20 copies/mL).

Alanine aminotransferase, aspartate aminotransferase, HBV infection-related markers (surface antigen [HBsAg], antibodies against HBsAg [anti-HBs], antibodies against core antigen [anti-HBc IgG and IgM] and HBV DNA by real-time PCR) and HCV markers (antibodies against HCV [anti-HCV] by enzyme immunoassay and recombinant immunoblot assay, and HCV RNA by real-time PCR) were analyzed. Recombinant immunoblot assay was used to confirm infection when the antibody detection was near the cutoff value for positive results.

All serological assays (HBsAg, anti-HBc IgG and anti-HBc IgM, anti-HBs and anti-HCV) were performed by commercial electrochemiluminescence enzyme immunoassays, including the 3600 Vitros System (Ortho Clinical Diagnostics-Johnson & Johnson, Dorchester, NY), Advia Centaur (Siemens, Munich, Germany) and Elecsys (Roche Diagnostics), which had similar analytical characteristics (sensitivity and specificity).

HBV-DNA and HCV-RNA levels (VL) were determined by real-time PCR technology, mainly on an AmpliPrep COBAS TaqMan system (Roche Diagnostics GmbH) with a lower limit of quantification of 20 IU/mL for HBV-DNA and 15 IU/mL for HCV-RNA and a lower limit of detection of <10/IU mL for both methods. The Abbot Real-Time HBV and HCV Assay (Chicago, IL), which has similar analytical characteristics, was used in 1 center.

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The diagnosis of confirmed OBI was established on HBV-DNA detection in the serum of HBsAg-negative patients, with or without other serologic markers of previous viral exposure.[4] We defined possible OBI as positive testing to anti-HBc IgG alone (anti-HBc alone serostatus) or to both anti-HBc and anti-HBs but no HBV-DNA detected. HBV-DNA detection in liver samples was not performed in any case. A case of seronegative or serosilent HCV infection was defined as simultaneous anti-HCV negativity and detectable (≥15 IU/mL) HCV-RNA VL.[3]

Results are expressed as the median and interquartile range for quantitative variables and as a percentage for qualitative variables. When antiretroviral drugs were analyzed as risk factors, naive patients were excluded. Comparisons between quantitative variables were performed with the Mann–Whitney *U* test. The Fisher exact test was used to compare categorical variables. Significance was set at a *P* value of <0.05. Statistical analyses were performed using Stata/SE 11.2 (StataCorp., College Station, TX).

RESULTS

Of 838 HIV-infected pediatric patients, 254 were evaluated (30% patients from the total of the cohort, 18% of centers). No significant differences were observed when comparing our population with the general cohort.[5] Median age was 14.4 years (interquartile range: 9.2–16.7), 55.6% of participants were female, and 69.3% were white. In 95% of patients, the route of HIV acquisition was mother-to-child transmission. Maternal serostatus was positive in 3.9% for HBV and in 24.4% for HCV; however, data regarding HBV and HCV infection serostatus were missing for 44.8% and 40.6% of the mothers, respectively.

Plasma HIV-VL was undetectable in two thirds, and median CD4+ T-cell count was 840/mm^3 (interquartile range: 577–1117). Almost one third of patients met the criteria for AIDS. Among patients on highly active antiretroviral therapy, ever/current exposure to drugs with anti-HBV activity were 91% and 30% for lamivudine/emtricitabine and tenofovir disoproxil fumarate, respectively.

Almost 90% of patients had completed the HBV vaccination schedule, but only one half of them achieved protective anti-HBs levels. No association was found between responsiveness and CD4+ T-lymphocyte nadir counts <200 cells/mm^3, higher VL or age.

Three (1.3%) patients were chronically infected with HBV at inclusion (HBsAg+, anti-HBc IgM+, anti-HBs−), HBV-DNA was negative but all of them received HIV/HBV-active drugs. No cases of acute HBV infection were detected.

Six possible OBI cases (2.4%; 95% confidence interval: 0.8–5.1), but no confirmed case were detected: 2 patients showed an anti-HBc alone pattern (0.8%) whereas 4 cases were both anti-HBc and anti-HBs positive (1.6%). HBV-DNA was undetectable in all cases and only 1 anti-HBc/anti-HBs-positive patient showed mild elevation of alanine aminotransferase and aspartate aminotransferase plasma levels (46 and 52 IU/mL, respectively). Five of these 6 patients were currently receiving at least 1 antiretroviral drug with activity against HBV, and did not show significantly lower CD4+ T-lymphocyte counts or higher plasma HIV-VL when compared with chronically HBV-infected patients.

Fifteen (6%) patients were coinfected with HCV when evaluated by conventional serological screening methods. Two of them had spontaneously cleared HCV infection. All of them were HBV-DNA negative. Triple HCV-HBV-HIV coinfection was not observed in any patient.

Seronegative HCV infection was detected in 2 patients (0.8%; 95% confidence interval: 0.1–2.8), both of whom showed elevated alanine aminotransferase plasma levels (51 and 77 IU/mL, respectively). There were no differences in HCV-RNA levels between anti-HCV positive and anti-HCV negative cases.

Data from possible OBI and seronegative HCV-infected patients are summarized in Table 1.

DISCUSSION

Although remarkable strides have been made in understanding the natural history and pathogenesis of viral hepatitis, there is still a lack of information regarding occult hepatitis infection, especially in children.

We found no cases of confirmed OBI based on positive HBV-DNA analysis, the only reliable noninvasive diagnostic

TABLE 1. Main Features of Patients With Possible Occult HBV Infection and Seronegative HCV Infection

	Possible OBI (2.4%)						Seronegative HCV Infection (0.8%)	
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
Age, yr	7	16	13	11	14	15	16	16
Sex	M	F	F	F	F	M	M	M
Ethnicity	A	W	W	A	W	A	W	W
Transmission	T	V	V	V	V	V	V	V
Current CD4+ T cell count (/mm^3)	1088	574	750	910	466	734	814	920
HIV VL (cop/mL)	63	Undetect	Undetect	Undetect	88,500	Undetect	150	Undetect
AIDS criteria	No	Yes	No	Yes	No	No	Yes	Yes
ALT (IU/mL)	46	19	20	21	21	25	49	43
AST (IU/mL)	52	18	14	21	14	26	51	77
Anti-HBs IgG	Positive	Undetect	Positive	Positive	Undetect	Positive	Undetect	Undetect
Anti-HBc IgG	Positive	Positive	Positive	Positive	Positive	Positive	Undetect	Undetect
HBV DNA	Undetect	Undetect	Undetect	Undetect	Undetect	Undetect	Undetect	Undetect
Anti-HCV	Undetect	Undetect	Undetect	Undetect	Undetect	Undetect	Undetect	Undetect
HCV RNA (cop/mL)	Undetect	Undetect	Undetect	Undetect	Undetect	Undetect	5,450,000	45,000
HBV vaccine	C	C	C	C	C	C	I	C
3TC/FTC* exposure	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
TDF* exposure	No	Yes	No	Yes	No	No	Yes	No
Maternal HBV	Unk	Positive	Unk	Unk	Unk	Positive	Unk	Negative
Maternal HCV	Unk	Positive	Positive	Unk	Unk	Unk	Positive	Positive

*Current or ever exposure.

A indicates African; ALT, alanine aminotransferase; AST, aspartate aminotransferase; C, complete; W, white; F, female; FTC, emtricitabine; I, incomplete; M, male; T, transfusion; TDF, tenofovir disoproxil fumarate; 3TC, lamivudine; V, vertically infected; Undetect, undetectable; Unk, unknown.

marker of OBI in clinical practice. As OBI status is dependent on longlasting persistence of viral genomes in the hepatocytes,[2] analysis of liver extracts is considered the reference standard diagnostic method for OBI. Liver samples were not available in our study, however; hence, OBI cannot be definitely excluded in our patients. In addition, HBV-DNA detection in serum can fluctuate over time, another possible explanation for the absence of positive samples in our study.[6] A major limitation of the survey is that most patients with "possible occult HBV" were receiving antiretroviral treatment active against HBV (ie, lamivudine/emtricitabine and/or tenofovir disoproxil fumarate), which might have suppressed HBV replication below the detection limits. Thus, it is highly recommendable to perform the screening for viral hepatitis before starting on antiretroviral treatment, including HBV-DNA and HCV-RNA.

Using the definitions in the consensus recommendations[4] based on the HBV antibody profile, HBV infection markers may be negative in up to 20% of patients, possibly due to progressive loss of specific antibodies.[2] In our opinion, this justifies implementation of HBV-DNA detection in the regular screening methods for HBV infection in HIV-infected children, as other authors have suggested.[4] On the other hand, false OBI results can also occur, with positive HBV-DNA and negative HBsAg caused by viral escape mutants that express a modified HBsAg not (or poorly) recognized by commercially available detection assays.[2]

The risk of OBI associated with the "anti-HBc alone" serological profile has been extensively demonstrated, and an antibody response to HBc can be considered a surrogate marker of OBI.[8] This was the case in 2 of 6 patients with possible OBI in our study.

In our study, the prevalence of HCV seronegative infection (0.8%) was low in comparison with previous studies,[2] a fact that may be related to the good overall immunological status of our cohort or the development of immune tolerance in the first years of life.

In 2 cases, anti-HCV was positive and HCV-RNA undetectable, which could indicate cleared infection. In 1 of them, HCV-RNA remained negative along subsequent assessment; the other patient was lost to further follow-up. As was reported by García et al,[7] follow-up sampling is strongly recommended, and we suggest checking seronegative patients for viremia on at least 2 time points, 6 months apart.

The novelty of this study is that it is the first to search for occult hepatitis coinfection in a large cohort of HIV-infected pediatric patients. The cross-sectional design is an obvious limitation, but it can be considered a snapshot of the current prevalence of occult hepatitis infection among children living with HIV in our country.

In conclusion, our data reinforce the need for the recent Spanish pediatric HIV guidelines recommendation to implement occult hepatitis screening (sensitive molecular assays for both HBV-DNA and HCV-RNA) in the routine care of these children.[9,10] Further work is needed to clarify the clinical significance of occult hepatitis infection and its pathogenic consequences in this unique growing population.

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