Hepatitis B, C, and D and HIV Infections among Immigrants from Equatorial Guinea Living in Spain

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Abstract. A total of 1,220 subjects from Equatorial Guinea living in Spain (median age = 41 years; 453 male and 767 female) was examined for antibodies to human immunodeficiency virus (HIV) and Hepatitis B (HBV), C (HCV), and D (HDV) viruses. Extracted RNA and DNA from the positive samples were used to quantify viral load. The prevalence of HIV antibodies, HCV RNA, and HBV surface antigen (HBsAg) was 10.8% ($N = 132$), 11.6% ($N = 141$), and 7.9% ($N = 96$), respectively. The most prevalent HIV variant was CRF02_AG (38.5%; $N = 40$). HCV genotype 4 (60%; $N = 36$) and HBV genotype A3 (32%; $N = 8$) were the hepatitis variants most frequently found. Superinfection with HDV was seen in 20.9% ($N = 24$) of HBsAg carriers. A control group of 276 immigrants from other sub-Saharan countries showed similar rates of HIV and HBsAg, although no HCV cases were found. Immigrants constitute a major source of HIV and hepatitis viruses in Spain; therefore, it is important that control measures are intensified.

INTRODUCTION

Population mobility poses important health risks, specifically in the spread of viral diseases. However, standardized routine screening of incoming immigrants and the delivery of medical care is often neglected.¹ In 2008, Spain was the European Union member state recording the largest number of immigrants.² In particular, citizens from The Republic of Equatorial Guinea (EG), a former Spanish colony located in West Central Africa, accounted for the majority of sub-Saharan Africans attended in many Spanish hospitals.^{3–6} Information about the seroprevalence of human immunodeficiency virus (HIV) and viral hepatitis in the immigrant population from EG living in Spain is scarce. Studies conducted so far are very heterogeneous and often include immigrants from other African countries.

In the present study, we sought to analyze the prevalence of HIV and viral hepatitis in a large number of immigrants from EG living in Spain. We also examined the extent of viral coinfections and the distribution of different genotypes and subtypes. We compared the data obtained in this population with those data collected in a control group of individuals from other sub-Saharan African countries with no historical link to Spain.

METHODS

Study population. The study was conducted at the Unit of Tropical Medicine, Hospital Carlos III, Madrid, Spain. All consecutive migrants from sub-Saharan Africa seen for first consultation between January of 2002 and December of 2008 ages > 16 years and previously tested for HIV, HCV, and HBV were retrospectively evaluated.

Individuals were grouped according to their origin (EG versus the rest) in subsequent comparisons. The main demographic information was recorded in an electronic case report form specially designed for this study.

Ethical statement. The Hospital Carlos III ethics committee approved this retrospective study and waived the informed consent requirement.

HIV. Two enzyme immunoassays (EIAs) were used for the detection of antibodies to both HIV-1 and HIV-2 (AxSYM; Abbott, Chicago, IL and Genscreen; Bio-Rad, Marnes la Coquette, France). Reactive samples were confirmed by Western blot (New LAV BLOT I; Bio-Rad) and a line immunoassay (Pepti-LAV; Bio-Rad) that distinguishes between HIV-1 and HIV-2 infection. Plasma HIV-RNA was quantified using bDNA technologies (HIV Quantiplex v3.0; Bayer Diagnostics or Versant HIV-1 RNA; Siemens, Barcelona, Spain). To determine HIV-1 subtypes, the HIV *pol* region, including the complete protease and part of the reverse transcriptase regions, was amplified using Trugene (Siemens) or ViroseqTM HIV-1 Genotyping System (Celaera Diagnostics, Alameda, CA) with codons 1–247 or 1–335, respectively. HIV subtyping was inferred using the REGA 2.0 genotyping tool available at <http://www.bioafrica.net/subtypetool/html>.

CD4+. CD4+ T-cell counts were determined by flow cytometry using specific labeled monoclonal antibodies (Beckman Coulter, Madrid, Spain). To evaluate HIV and viral hepatitis markers, different tests were used along the years.

Hepatitis C virus. Hepatitis C virus (HCV) antibodies were tested using a commercial EIA (AxSYM; Abbott) and further confirmed and genotyped using a line immunoassay (INNO-LIA HCV Ab III; Innogenetics, Ghent, Belgium). To confirm active HCV replication, serum HCV-RNA levels were analyzed by two methods (Cobas Monitor HCV; Roche Diagnostics and Abbott Real Time HCV; Abbott).

Hepatitis B virus. Serum Hepatitis B virus (HBV) markers were examined using EIA (AxSYM; Abbott). Serum HBV-DNA was measured using three quantitative polymerase chain reaction (PCR) assays (Cobas Amplicor HBV Monitor; Roche Diagnostics, Cobas Taqman; Roche Diagnostics, and Abbott Real Time HBV; Abbott). To standardize detectable thresholds values, the 200 copies/mL cutoff was taken for subsequent analyses. In addition, because the units of HBV-DNA measurements obtained by HBV Monitor were given in copies per milliliter, results from years 2002 to 2004 were not taken into account for quantitative analysis of HBV viral

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load. HBV genotyping was carried out using a commercial line probe assay, INNOLiPA HBV Genotype (Innogenetics, Ghent, Belgium). HBV-infected patients with normal aminotransferase (ALT) and undetectable HBV-DNA were considered inactive carriers.

Hepatitis D virus. Total Hepatitis D virus (HDV) antibodies were analyzed in those HBV surface antigen+ (HBsAg+) patients using an EIA (Radim Iberica, Barcelona, Spain).

Hepatic biomarkers. ALT and aspartate aminotransferase (AST) markers of hepatocellular injury were measured using a commercially available kit.

Statistical analysis. Categorical variables were reported as percentages, and continuous variables were reported as medians with interquartile ranges (IQRs). The χ^2 or Fisher's exact tests were used to detect significant differences between proportions. A *P* value < 0.05 was considered statistically significant. All data were analyzed using the SPSS v15.0 software package.

RESULTS

The whole study population included 1,493 individuals, of whom 1,220 (81.6%) were from EG and 276 (18.4%) from other sub-Saharan African countries, including 35 different nationalities. The five most common non-EG countries represented were Nigeria (*N* = 62), Cameroon (*N* = 42), Senegal (*N* = 29), Angola (*N* = 17), and Mali (*N* = 16). The median age of immigrants was 39 years (IQR = 29–51), and women represented 57.4% of individuals. The main demographics of the study population are recorded in Table 1.

HIV infection. The overall prevalence of HIV infection was 10.8% (Table 2). It did not differ significantly comparing immigrants from EG with immigrants from other sub-Saharan countries (10.8% versus 8.3%, respectively). There were no significant differences in the yearly prevalence of HIV over time, regardless country of origin. The median age of HIV-infected individuals and the proportion of women were significantly higher in subjects coming from EG than other sub-Saharan countries (Table 3).

Most HIV patients harbored HIV type 1 (mostly non-B subtypes). Two patients from EG were infected by HIV type 2. One individual from Guinea Bissau was coinfecting with HIV-1 and HIV-2. Overall, the most common non-B variant of HIV-1 was CRF02_AG. The proportion of HIV patients coinfecting with HBV or HCV did not differ comparing EG with other sub-Saharan countries. The median percentage of CD4+ counts and plasma HIV-RNA was also similar. Despite

TABLE 1

Main demographics of the immigrant population according to their origin

	Equatorial Guinea (<i>N</i> = 1,220)	Other sub-Saharan countries (<i>N</i> = 276)	<i>P</i>
Female sex (<i>n</i> , %)	767 (62.9)	91 (33)	< 0.001
Age (in years) median (IQR)	41 (30–53)	33 (27–39)	< 0.001
Age distribution in years (<i>n</i> , %)			
< 21	66 (5.4)	16 (5.8)	< 0.001
21–30	240 (19.7)	92 (33.3)	
31–40	288 (23.6)	109 (39.1)	
41–50	262 (21.5)	41 (14.9)	
> 50	364 (29.8)	19 (6.9)	
Time in Spain (in weeks) median (IQR)	4 (1–312)	28 (2–144)	< 0.001

TABLE 2

Serologic status of the immigrant population according to their origin

	Equatorial Guinea (<i>N</i> = 1,220)	Other sub-Saharan countries (<i>N</i> = 276)	<i>P</i>
HIV-positive (<i>n</i> , %)	132 (10.8)	23 (8.3)	0.221
HBV status (<i>n</i> , %)			
HBsAg+	96 (7.9)	30 (10.9)	0.105
Past infection	821 (67.3)	127 (46)	< 0.001
Negative markers	97 (8)	74 (26.8)	< 0.001
Vaccinated	52 (4.3)	19 (6.9)	0.063
Isolated anti-HBc	154 (12.7)	26 (9.4)	0.126
HCV status (<i>n</i> , %)			
HCV Ab+ and HCV-RNA+ (%)	141 (11.6)	0 (0)	< 0.001
HCV Ab+ and HCV-RNA neg (%)	93 (7.6)	2 (0.7)	< 0.001
HCV Ab neg (%)	986 (80.4)	274 (99.3)	< 0.001

HCV Ab and HCV-RNA = hepatitis C virus antibodies and PCR.

the fact that two-thirds of patients had CD4+ T cells/mm³ lower than 350, less than 10% of subjects were on antiretroviral therapy (Table 3).

HCV. HCV antibodies were recognized in 15.7% of individuals, of whom 60.2% were viremic (9.4% of the total population). Patients from EG showed HCV seropositivity in 19.2% of cases, and 11.6% were viremic. No cases of viremic HCV infection were found in subjects from other sub-Saharan countries (Table 2). The HCV seroprevalence did not change significantly along the study period and was similar for men and women. The median age of subjects with chronic HCV from EG was significantly higher than in those subjects with negative HCV markers (58 versus 38 years, respectively; *P* < 0.001). For EG immigrants ages > 50 years, HCV seroprevalence and HCV viremia were 45.3% and 28.8%, respectively (Figure 1).

Serum HCV-RNA could be measured in 47 HCV-seropositive patients from EG. The median HCV-RNA titer was 450,138 IU/mL (IQR = 99,900–1,770,000). The number of patients with values < 600,000 IU/mL was 53.2%. HCV genotype was available in 60 patients from EG, being distributed as follows: 36 patients with genotype 4 (60%), 21 patients with genotype 1 (35%), 2 patients with genotype 3 (3.3%), and 1 patient with genotype 2 (1.7%). AST and ALT levels were normal in 77.7% and 69.1% of patients, respectively, with chronic HCV.

HBV. A total of 126 individuals (8.4%) were HBsAg+ (Table 2): 7.9% in subjects from EG and 10.9% in subjects from other sub-Saharan countries (*P* = 0.10). The prevalence remained fairly stable along the study period in both groups. A majority of subjects HBsAg+ were younger than 40 years (Figure 1). In subjects from EG, the median age in HBsAg+ was significantly lower than in HBsAg-negative subjects (33 versus 42 years, respectively; *P* < 0.001). This difference was even more pronounced compared with chronic HCV patients from EG (*P* < 0.001).

Globally, the HBV prevalence was more than double in men than women (12.5% versus 5.4%, respectively; *P* < 0.001), although the median age of HBsAg+ males and females did not differ. This difference in sex remained significant when analyzing separately patients from EG (11.7% of males versus 5.6% of females; *P* < 0.001) and other sub-Saharan countries (14.6% of males versus 3.3% of females; *P* = 0.004).

Serum HBV-DNA could be measured in 76 of 126 HBsAg+ Africans and was detectable in 40 (52.6%) subjects. The proportion of patients with detectable HBV-DNA was numerically higher but not statistically significant in EG and the rest

TABLE 3
Differences among HIV-infected immigrants according to their origin

	Equatorial Guinea (N = 132)	Other sub-Saharan countries (N = 23)	P
Female sex (n, %)	80 (60.6)	7 (32.4)	0.007
Age (in years) median (IQR)	41 (33–47)	32 (27–38)	< 0.001
HIV type 1 (n, %)	130 (98.5)	22 (95.7)	0.38
HIV type 2 (n, %)	2 (1.5)	0 (0)	
HIV-1 and -2 coinfection (n, %)	0 (0)	1 (4.3)	
HIV-1 subtype variants (n, %)			
B	3 (2.9)	1 (5.6)	0.47
CRF02_AG	40 (38.5)	8 (44.4)	0.63
G	14 (13.5)	3 (16.7)	0.71
A	13 (12.5)	0 (0)	0.21
C	10 (9.6)	0 (0)	0.17
F	4 (3.8)	0 (0)	
CRF11_cpx	4 (3.8)	1 (5.6)	
D	3 (2.9)	1 (5.6)	
CRF06_cpx	3 (2.9)	3 (16.7)	0.041
CRF14_BG	2 (1.9)	0 (0)	
H	2 (1.9)	0 (0)	
J	2 (1.9)	0 (0)	
CRF01_AE	0 (0)	1 (5.6)	
Other subtypes	4 (3.8)	0 (0)	
HIV-HCV coinfection	13 (9.8)	0 (0)	0.21
HIV-HBV coinfection	11 (8.3)	3 (13)	0.43
HBV-HCV-HIV coinfection	0 (0)	0 (0)	
Patients on ARV treatment (n, %)	13 (9.8)	1 (4.8)	0.69
CD4 count (cells/ μ L) median (IQR)	292 (160–458)	202 (64–318)	0.010
Percentage of CD4 median (IQR)	16 (10–24)	17.5 (4.7–21.7)	0.50
CD4 count < 350 cells/ μ L (n, %)	77 (62.1)	18 (90)	0.02

HIV subtyping was performed in 122 individuals: 104 individuals from Equatorial Guinea and 18 individuals from other sub-Saharan countries. CD4 counts were available for 144 patients: 120 patients from Equatorial Guinea and 20 patients from other sub-Saharan countries. ARV = antiretroviral.

(48.3% versus 66.7%, respectively; $P = 0.17$). The median HBV-DNA in viremic subjects was 4,000 IU/mL (IQR = 1,000–436,583), being 3,000 IU/mL in patients from EG and 5,791 IU/mL in patients from other African countries ($P = 0.29$). Globally, 59.2% of chronic HBV patients had HBV-DNA < 1,000 IU/mL (63.8% in EG and 44.4% in other Africans; $P = 0.14$). In addition, 44% of HBsAg+ patients had both HBV-DNA < 1,000 IU/mL and normal ALT (44.8% in EG and 41.2% in other Africans; $P = 0.79$). Interestingly, 8 of 31 (25.8%) of these patients with low HBV viremia and normal ALT tested positive for HDV antibodies.

In 25 HBV viremic patients from EG, HBV genotype distribution was as follows: 15 patients had genotype A (8 patients had A3, 3 patients had A1, and 4 patients had A untypable), 6 patients had genotype E, and 1 patient had genotype D2. Two patients were A/F coinfecting, and one patient was A/D

coinfecting. In nine viremic patients from other African countries, HBV genotype distribution was as follows: four patients had genotype A (three patients had A3 and one patient had A1), four patients had genotype E, and one patient had genotype D2. Globally, genotypes A (64.7%) and E (29.4%) accounted for the majority of HBV infections.

Serum HBeAg could be tested in 123 HBsAg+ patients and was positive in only 6 (4.9%) patients. No differences were found comparing EG with the rest (4.3% versus 6.7%; $P = 0.633$). Serum HBV-DNA was performed in five HBeAg+ patients and detectable in all of them. Median values in HBeAg+ versus HBeAg– subjects were 4,000 (IQR = 1,351–11,000,000) versus 100 (IQR = 8–3,000) IU/mL ($P = 0.061$).

AST and ALT levels were within normal values in 77% and 68.9% of chronic HBV patients. ALT levels correlated weakly with serum HBV-DNA ($r = 0.266$, $P = 0.044$). This correlation was stronger in HDV-seronegative patients ($r = 0.418$, $P = 0.006$) and absent in HDV-positive patients.

Overall, 63.4% of the whole study population had serum markers of past HBV exposure. Isolated anti-HBc seropositivity was recognized in 12%, whereas 4.7% had been vaccinated; 11.4% were negative for all HBV markers. The proportion of patients with past HBV infection increased with age (Figure 2). Patients from African countries other than EG were significantly more likely to be negative for all HBV markers (Table 3).

Hepatitis- δ . HDV antibodies were tested in 115 HBsAg+ patients and positive in 24 (20.9%) patients. The HDV seroprevalence tended to be higher in immigrants from EG (21/86, 24.4%) than in the rest of Africans (3/29, 10.3%; $P = 0.12$). Median serum HBV-DNA was significantly lower in HDV-positive than -negative patients with HBsAg (10 [IQR = 6–500] versus 620 [IQR = 20–5197] IU/mL; $P = 0.0029$).

FIGURE 1. Prevalence of chronic HBV, HCV, and HIV infection in 1,220 immigrants from Equatorial Guinea according to age.

FIGURE 2. Serum HBV markers in 1,496 sub-Saharan African immigrants according to age.

Likewise, there was a trend to a higher proportion of patients with undetectable HBV-DNA in HDV-positive than -negative immigrants (66.7% versus 40.7%; $P = 0.056$).

Coinfection with HIV and hepatitis viruses. The prevalence of HIV-HCV, HIV-HBV, and HBV-HCV coinfections in the 1,220 individuals from EG was 1.1%, 0.9%, and 0.2%, respectively. No cases of triple HIV-HBV-HCV coinfection were found. The rates of HIV-HCV and HIV-HBV coinfection were 9.8% in EG and 8.3% in the rest (Table 3). HDV was found in 2 of 10 patients with HIV-HBV coinfection. HIV-HCV-coinfected patients were significantly older than HIV-HBV-coinfected patients (median age 57 versus 37 years, $P < 0.001$). Only one HIV-HBV-coinfected patient was on antiretroviral therapy. She was a 38-year-old woman who was receiving lamivudine, stavudine, and efavirenz for the previous 6 months. Her plasma HIV-RNA was undetectable, but serum HBV-DNA was 4,000 IU/mL.

DISCUSSION

We found a high prevalence of HIV and viral hepatitis testing 1,220 immigrants from EG attended at our institution during the last decade, with a wide variety of viral genotypes and subtypes, most of which are rarely seen in native Europeans. Up to 10.8% of EG immigrants were HIV-positive, a rate higher than the rate found in other studies that examined sub-Saharan immigrants in Spain, which reported figures ranging from 4%⁴ to 6.1%.³ Estimates of the global HIV prevalence in Africa were 5.2% in the year 2008.⁷ Moreover, in previous studies conducted in EG, the rates of HIV infection were only as high as 0.6–1.23%,^{8,9} although the World Health Organization (WHO) has reported a fairly stable prevalence of 3.5% between 2001 and 2007 in EG adults.¹⁰ Clearly, immigrants from the tropics in this study do not accurately reflect the epidemiology of resident populations in their original countries. An overestimation of HIV infection among immigrants may reflect the search for care by subjects already aware of their HIV-positive status; seeking a developed country where antiviral therapy is given without charge may have been one reason for their migration. Indeed, CD4 counts were < 350 cells/ μ L in more than two-thirds of HIV-infected immigrants in our study, indicating long-term

infection. This finding is congruous with the low antiretroviral therapy coverage rate reported in EG.^{10,11}

Women constituted a high proportion of HIV patients among immigrants from EG, which generally occurs in Africa, where women account for 60% of estimated HIV infections.⁷ Moreover, most HIV-1 patients carried non-B subtypes, with CRF02_AG being the most common variant in patients from either EG or other African countries. CRF02_AG is well-known to predominate in West and parts of West Central Africa.¹² A wide variety of other HIV-1 subtypes were found. Although immigration has already been related with a widespread of HIV-1 non-B subtypes in Spain,¹³ no major impact on treatment outcomes or diagnostic procedures are of concern.¹⁴

The WHO has estimated an overall prevalence of 5.3% for HCV infection in the African region.¹⁵ We found an unexpected higher rate of chronic HCV in EG immigrants, particularly in those immigrants older than 50 years. The overall HCV seroprevalence found in individuals from EG (19.2%) was roughly double what has been found in other studies conducted in sub-Saharan immigrants living in Spain.^{3,4} Other than the burden, the wide HCV diversity was striking, although information on this aspect was so far scarce for EG.^{8,16} The HCV prevalence reported in countries bordering EG was 9.8% in Gabon and 13.8% in Cameroon, rising up to 30–50% among elderly people.^{15,17,18} Few HCV infections in Africans could be attributed to injecting drug use and therefore, exposure to contaminated blood transfusions, medical equipment, or needle sticks, including those instruments used by traditional healers, could be responsible for most cases. Prior studies in Africa have claimed that medical interventions and/or traditional practices (i.e., circumcision) could have been associated with massive transmission of HCV decades ago.¹⁹ Additional studies are needed to elucidate if specific medical interventions could have triggered the HCV epidemic in EG. A striking absence of HCV antibodies was detected in the control group of immigrants coming from other sub-Saharan African countries, which also could be attributed to the lack of injecting drug users in this group.

HCV genotypes 4 and 1 accounted for 60% and 35% of chronic HCV cases, respectively. A high prevalence of HCV genotype 4 has already been reported in countries neighboring EG.^{17,18} Given the low response to current therapy with

peginterferon- α plus ribavirin in patients harboring HCV genotype 4 and that new antivirals for HCV, such as telaprevir or boceprevir, are not active against this variant,²⁰ an increasing number of these patients in Spain will be predicted to develop decompensated cirrhosis and liver cancer within the next years. Transaminases were within normal values in many of these subjects; nevertheless, the extent of liver fibrosis may be significant in a substantial proportion of them.²¹ Therefore, all African immigrants should be screened for viral hepatitis regardless of their transaminases levels, and liver fibrosis should be assessed in those immigrants with markers of viral hepatitis using either liver biopsy or non-invasive tools.^{21,22}

The prevalence of chronic HBV in our immigrant population from EG was 7.9% (Table 2). This prevalence is in agreement with rates reported by others in sub-Saharan immigrants coming to Spain^{3,4,6} and estimates in EG.⁸ The median age of HBsAg+ patients was 33 years, significantly lower than the age of chronic HCV patients (58 years), presumably because most Africans are exposed to HBV early in life through vertical or childhood horizontal transmission.²³ Interestingly, HBV infection was significantly more frequent in men than women (12.5% versus 5.4%, respectively). This male preponderance has also been noticed in other studies conducted in EG as well as other African countries^{8,24,25} and among sub-Saharan immigrants to Europe.^{4,26} It contrasts with the higher proportion of women with HIV compared with men in Africa. However, an explanation for this discrepancy remains unknown.

HBV variants A3 and E are circulating throughout Central and West Africa.^{27,28} Accordingly, most chronic HBV patients in our study harbored these HBV genotypes, confirming that they are also the most prevalent in EG. Given that differences in pathogenicity have been noticed for distinct HBV genotypes,²⁹ our results add an important piece of information about the molecular epidemiology of HBV in the region. As in chronic HCV patients, nearly three-quarters of subjects with chronic HBV in our study had ALT and AST within normal values. Serum HBeAg+ was uncommon, and HBV-DNA was below the limit of detection of 1,000 IU/mL in around two-thirds of Africans. Altogether, more than 40% of chronic HBV patients had both normal ALT and undetectable HBV-DNA, suggesting a high rate of inactive HBV carriers, for whom the risk of clinical progression and need for antiviral therapy is low.²⁹

Hepatitis- δ is endemic in Central Africa.²⁸ Accordingly, one-quarter of HBsAg+ patients from EG were superinfected by HDV. Viral interference explained that HBV-DNA was generally suppressed in most HDV patients.³⁰ Despite the reported worse outcome of δ -hepatitis,³⁰ up to 54% of our HDV patients exhibited ALT values within normal range, suggesting a lower pathogenicity than expected. Additional studies should clarify the natural history of liver disease in this population.

There is very limited information regarding HIV and viral hepatitis coinfections in EG. HBV and HCV were recognized in 8% and 10%, respectively, of EG immigrants. Because HIV accelerates the course of liver diseases in patients with chronic HBV or HCV infections, attention and when possible, treatment of these conditions must be prioritized. Caution must be used when providing antiretroviral drugs with anti-HBV activity, and the use of lamivudine as a single anti-HBV agent, when possible, must be avoided given the high risk of

failure, selection of HBV resistance and cross-resistance to other anti-HBV agents, and emergence of HBV escape mutants that may eventually evade serological diagnosis and vaccine protection.³¹ Only one HIV-HBV-coinfecting patient was on a lamivudine-containing regimen in our series. She harbored elevated serum HBV-DNA, suggesting that selection of resistance already had occurred. With the scaling-up of antiretroviral treatment in Africa, this phenomenon is likely to increase in the future.

A limitation of our study is that patients from EG attending our institution were disproportionately represented by women and elderly people, which might not represent the healthy asymptomatic immigrant population that is generally comprised of young men.³²

In conclusion, immigrants constitute a major source of HIV and hepatitis viruses in Spain; therefore, it is important that screening of these viruses and counseling of the patients should be prioritized in this population to prevent additional transmissions in Spain.

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REFERENCES

1. Gushulak BD, MacPherson DW, 2004. Globalization of infectious diseases: the impact of migration. *Clin Infect Dis* 38: 1742–1748.
2. Oblakf Lander A, 2011. *Immigration to EU Member States down by 6% and Emigration Up by 13 % in 2008. Eurostat Statistics in Focus 1/2011*. Available at: http://epp.eurostat.ec.europa.eu/cache/ITY_OFFPUB/KS-SF-11-001/EN/KS-SF-11-001-EN.PDF. Accessed February 12, 2012.
3. López-Vélez R, Huerga H, Turrientes MC, 2003. Infectious diseases in immigrants from the perspective of a tropical medicine referral unit. *Am J Trop Med Hyg* 69: 115–121.
4. Manzardo C, Treviño B, Gómez i Prat J, Cabezas J, Monguí E, Clavería I, Luis Del Val J, Zabaleta E, Zarzuela F, Navarro R, 2008. Communicable diseases in the immigrant population attended to in a tropical medicine unit: epidemiological aspects and public health issues. *Travel Med Infect Dis* 6: 4–11.
5. Toro C, Jiménez V, Rodríguez C, Del Romero J, Rodés B, Holguín A, Alvarez P, García-Campello M, Gómez-Hernando C, Guelar A, Sheldon J, de Mendoza C, Simón A, Soriano V, 2006. Molecular and epidemiological characteristics of blood-borne virus infections among recent immigrants in Spain. *J Med Virol* 78: 1599–1608.
6. Monge-Maillo B, Jiménez BC, Pérez-Molina JA, Norman F, Navarro M, Pérez-Ayala A, Herrero JM, Zamarrón P, López-Vélez R, 2009. Imported infectious diseases in mobile populations, Spain. *Emerg Infect Dis* 15: 1745–1752.
7. Joint United Nations Programme on HIV/AIDS; World Health Organization, 2009. *AIDS Epidemic Update December 2009*.

- Available at: http://data.unaids.org/pub/report/2009/jc1700_epi_update_2009_en.pdf. Accessed February 12, 2012.
8. Basaras M, Santamaría A, Sarsa M, Gutiérrez E, de Olano Y, Cisterna R, 1999. Seroprevalence of hepatitis B and C, and human immunodeficiency type 1 viruses in a rural population from the Republic of Equatorial Guinea. *Trans R Soc Trop Med Hyg* 93: 250–252.
 9. Jimoh AAG, 2004. Prevalence of HIV antibodies amongst apparently healthy pregnant women in Mongomo, Guinea Equatoria. *Niger J Med* 13: 114–117.
 10. UNAIDS/WHO Working Group on Global HIV/AIDS and STI, 2008. *UNAIDS/WHO Epidemiological Fact Sheets on HIV and AIDS, 2008 Update. Equatorial Guinea*. Available at: http://apps.who.int/globalatlas/predefinedReports/EFS2008/full/EFS2008_GQ.pdf. Accessed February 12, 2012.
 11. GlobalHealthFacts.org, 2012. *Antiretroviral Therapy Coverage Rate in Low- and Middle-Income Countries*. Available at: <http://www.globalhealthfacts.org/data/topic/map.aspx?ind=11>. Accessed December 23, 2012.
 12. Thomson MM, Nájera R, 2005. Molecular epidemiology of HIV-1 variants in the global AIDS pandemic: an update. *AIDS Rev* 7: 210–224.
 13. Treviño A, Soriano V, Rodríguez C, Arredondo M, Rivas P, Herrero-Mendoza D, Parra P, del Romero J, Anta L, Puente S, de Mendoza C, 2011. Changing rate of non-B subtypes and coinfection with hepatitis B/C viruses in newly diagnosed HIV type 1 individuals in Spain. *AIDS Res Hum Retroviruses* 27: 633–638.
 14. Geretti AM, Harrison L, Green H, Sabin C, Hill T, Fearnhill E, Pillay D, Dunn D; UK Collaborative Group on HIV Drug Resistance, 2009. Effect of HIV-1 subtype on virologic and immunologic response to starting highly active antiretroviral therapy. *Clin Infect Dis* 48: 1296–1305.
 15. Madhava V, Burgess C, Drucker E, 2002. Epidemiology of chronic hepatitis C virus infection in sub-Saharan Africa. *Lancet Infect Dis* 2: 293–302.
 16. Bracho MA, Carrillo-Cruz FY, Ortega E, Moya A, González-Candelas F, 2006. A new subtype of hepatitis C virus genotype 1: complete genome and phylogenetic relationships of an Equatorial Guinea isolate. *J Gen Virol* 87: 1697–1702.
 17. Ndong-Atome GR, Makuwa M, Ouwe-Missi-Oukem-Boyer O, Pybus OG, Branger M, Le Hello S, Boye-Cheik SB, Brun-Vezinet F, Kazanji M, Roques P, Bissler S, 2008. High prevalence of hepatitis C virus infection and predominance of genotype 4 in rural Gabon. *J Med Virol* 80: 1581–1587.
 18. Nerrienet E, Pouillot R, Lachenal G, Njouom R, Mfoupouendoun J, Bilong C, Mauciere P, Pasquier C, Ayoub A, 2005. Hepatitis C virus infection in cameroon: a cohort-effect. *J Med Virol* 76: 208–214.
 19. Pépin J, Lavoie M, Pybus OG, Pouillot R, Foupouapouognigni Y, Rousset D, Labbé AC, Njouom R, 2010. Risk factors for hepatitis C virus transmission in colonial Cameroon. *Clin Infect Dis* 51: 768–776.
 20. Craxi A, Pawlotsky JM, Wedemeyer H, Bjoro K, Flisiak R, Fornis X, Mondelli M, Peck-Radosavljevic M, Rosenberg W, Sarrazin C, Jacobson I, Dusheiko G, 2011. EASL clinical practice guidelines: management of hepatitis C virus infection. *J Hepatol* 55: 245–264.
 21. Derbala MF, Amer AM, Almohanadi M, John A, Amin A, John A, Sharma M, Alkaabi SR, Al Dweik NZ, Pasic F, Yaqoob R, Butt MT, Shebl FM, 2011. Hepatitis C virus genotype 4 with normal transaminases: histological changes, schistosomiasis and response to treatment. *J Viral Hepat* 18: e258–e262.
 22. Rosen HR, 2011. Clinical practice. Chronic hepatitis C infection. *N Engl J Med* 364: 2429–2438.
 23. Edmunds WJ, Medley GF, Nokes DJ, O'Callaghan CJ, Whittle HC, Hall AJ, 1996. Epidemiological patterns of hepatitis B virus (HBV) in highly endemic areas. *Epidemiol Infect* 117: 313–325.
 24. Kew MC, 2008. Hepatitis B virus infection: the burden of disease in South Africa. *South Afr J Epidemiol Infect* 23: 4–8.
 25. Valente F, do Lago BV, de Castro CAV, de Almeida AJ, Gomes SA, Soares CC, 2010. Epidemiology and molecular characterization of hepatitis B virus in Luanda, Angola. *Mem Inst Oswaldo Cruz* 105: 970–977.
 26. Majori S, Baldo V, Tommasi I, Malizia M, Floreani A, Monteiro G, Ferrari A, Accordini A, Guzzo P, Baldovin T, 2008. Hepatitis A, B, and C Infection in a community of sub-saharan immigrants living in Verona (Italy). *J Travel Med* 15: 323–327.
 27. Kurbanov F, Tanaka Y, Fujiwara K, Sugauchi F, Mbanya D, Zekeng L, Ndembi N, Ngansop C, Kaptue L, Miura T, Ido E, Hayami M, Ichimura H, Mizokami M, 2005. A new subtype (subgenotype) Ac (A3) of hepatitis B virus and recombination between genotypes A and E in Cameroon. *J Gen Virol* 86: 2047–2056.
 28. Makuwa M, Mints-Ndong A, Souquière S, Nkoghe D, Leroy EM, Kazanji M, 2009. Prevalence and molecular diversity of hepatitis B virus and hepatitis delta virus in urban and rural populations in northern Gabon in central Africa. *J Clin Microbiol* 47: 2265–2268.
 29. Dienstag JL, 2008. Hepatitis B virus infection. *N Engl J Med* 359: 1486–1500.
 30. Rizzetto M, 2009. Hepatitis D: thirty years after. *J Hepatol* 50: 1043–1050.
 31. Sheldon J, Soriano V, 2008. Hepatitis B virus escape mutants induced by antiviral therapy. *J Antimicrob Chemother* 61: 766–768.
 32. Reher DS, Cortés Alcalá L, González Quiñones F, Requena M, Sánchez Domínguez MI, Sanz Gimeno A, Stanek M, 2008. *Informe Encuesta Nacional de Inmigrantes (ENI-2007)*. Available at: http://www.ine.es/daco/daco42/inmigrantes/informe/eni07_informe.pdf. Accessed February 12, 2012.