ORIGINAL ARTICLE

Nucleic Acid Testing to Detect HBV Infection in Blood Donors

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ABSTRACT

BACKGROUND

The detection of hepatitis B virus (HBV) in blood donors is achieved by screening for hepatitis B surface antigen (HBsAg) and for antibodies against hepatitis B core antigen (anti-HBc). However, donors who are positive for HBV DNA are currently not identified during the window period before seroconversion. The current use of nucleic acid testing for detection of the human immunodeficiency virus (HIV) and hepatitis C virus (HCV) RNA and HBV DNA in a single triplex assay may provide additional safety.

METHODS

We performed nucleic acid testing on 3.7 million blood donations and further evaluated those that were HBV DNA–positive but negative for HBsAg and anti-HBc. We determined the serologic, biochemical, and molecular features of samples that were found to contain only HBV DNA and performed similar analyses of follow-up samples and samples from sexual partners of infected donors. Seronegative HIV and HCV-positive donors were also studied.

RESULTS

We identified 9 donors who were positive for HBV DNA (1 in 410,540 donations), including 6 samples from donors who had received the HBV vaccine, in whom subclinical infection had developed and resolved. Of the HBV DNA–positive donors, 4 probably acquired HBV infection from a chronically infected sexual partner. Clinically significant liver injury developed in 2 unvaccinated donors. In 5 of the 6 vaccinated donors, a non-A genotype was identified as the dominant strain, whereas subgenotype A2 (represented in the HBV vaccine) was the dominant strain in unvaccinated donors. Of 75 reactive nucleic acid test results identified in seronegative blood donations, 26 (9 HBV, 15 HCV, and 2 HIV) were confirmed as positive.

CONCLUSIONS

Triplex nucleic acid testing detected potentially infectious HBV, along with HIV and HCV, during the window period before seroconversion. HBV vaccination appeared to be protective, with a breakthrough subclinical infection occurring with non-A2 HBV subgenotypes and causing clinically inconsequential outcomes. (Funded by the American Red Cross and others.)

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The transfusion of blood containing hepatitis B surface antigen (HBsAg) is associated with post-transfusion infection with hepatitis B virus (HBV). Blood that is free of HBsAg but has high-titer antibodies against hepatitis B core antigen (anti-HBc) in the absence of antibodies against hepatitis B surface antigen (anti-HBs) can also transmit HBV infection.[1,2] In 1986, screening for anti-HBc was implemented in the United States to reduce HBV transmission and as a surrogate marker for non-A, non-B hepatitis (i.e., hepatitis C virus [HCV]).[3,4] However, a small proportion of donors with anti-HBc in the absence of HBsAg have circulating HBV DNA and may have a risk of infectivity.[1-6]

Blood that is collected during the early window period of HBV infection is highly infectious, but this risk declines as anti-HBs develops.[5,7-12] The estimated residual risk of HBV infection from donations to the American Red Cross ranges from 1 in 280,000 to 1 in 357,000 donations.[13] After the introduction of nucleic acid testing for screening in minipools (pools of 6 to 16 donations), the estimated yield of HBV infection ranges from 1 in 830,000 to 1 in 2 million donations.[14] However, these estimates do not capture the possibility of HBV infection in vaccinated donors who have acute infection with low or no expression of HBsAg. HBV-seronegative but infected donors have been identified by means of nucleic acid testing at a rate of approximately 1 in 600,000 donations screened[15] but have not been further studied. Although the residual risk of transmission of HBV by transfusion has decreased progressively,[13,16,17] it remains higher than the estimated risks for human immunodeficiency virus (HIV) and HCV (1 in 1,467,000 donations and 1 in 1,149,000 donations, respectively).[18]

We conducted a study to evaluate the use of nucleic acid testing in determining the number of seronegative donors who have HBV DNA and to characterize these donors according to their risk factors and progression of molecular, serologic, and biochemical markers. We used a triplex test combining the detection of HBV DNA with that of HIV and HCV RNA. We also evaluated donors who were seronegative for HIV and HCV but had positive results on nucleic acid testing.

Methods

Screening Protocol

During 2008, the American Red Cross implemented prospective screening of all blood donations, using the Procleix Ultrio assay and the TIGRIS automated platform[10] (Gen-Probe and Novartis) at three of its five National Testing Laboratories. The American Red Cross collects about 42% of the U.S. blood supply from volunteer, unpaid donors in the continental United States and Puerto Rico. All donations were screened by nucleic acid testing of either individual donations or 16-sample minipools. Blood units that were released for transfusion were negative on triplex nucleic acid testing and for all standard blood-screening markers.[18] All Ultrio-reactive minipools were resolved to the reactive individual donation, followed by testing of the reactive individual donations (including donations screened individually by nucleic acid testing) by means of separate discriminatory assays for HBV, HIV, and HCV. Any individual donation with a positive result on nucleic acid testing was not used for transfusion (even if the donor was subsequently determined to have a false positive result), and associated donors were deferred from future donation. Seronegative donors with one or multiple reactive discriminatory tests were considered potentially infected donors for further study. For individual donations, the 95% lower limit of detection for the HBV portion of Ultrio is 10.4 IU per milliliter (approximately 50 copies per milliliter), as compared with approximately 30 copies per milliliter each for HIV and HCV.

The study protocol was approved by the institutional review board of the American Red Cross. Written informed consent for follow-up studies was obtained from donors who were reactive on nucleic acid testing and from their partners. The study was conducted in accordance with the provisions of the protocol.

HBV DNA Confirmation

Donors who were positive for HBV DNA on the Ultrio assay and seronegative for HBsAg (lower limit of detection, 0.10 ng per milliliter) and for total (IgM and IgG) anti-HBc (HBsAg and HBcore PRISM, Abbott Laboratories) were enrolled in

follow-up studies, which involved the collection of samples at intervals of 1 to 2 weeks until seroconversion to anti-HBc occurred. Each plasma unit from a reactive donation was retrieved for further testing.

Additional testing that was repeated on all index donations, as well as performed on each follow-up sample, included duplicate Ultrio nucleic acid testing of individual donations and discriminatory testing if reactivity was observed, followed by confirmation by means of polymerase-chain-reaction (PCR) assay with the use of COBAS AmpliScreen HBV Test (Roche) with the 1-milliliter extraction method (95% lower limit of detection, 5 IU per milliliter). In addition, the retrieved plasma units from each donation with reactivity on discriminatory testing were separated into aliquots and tested in replicates of 10 to confirm reactivity. Reactivity in at least 1 of the 10 replicate tests was required for confirmation of positive results; donations with no reactivity were considered to have false positive results.

Serologic testing was performed on retrieved plasma units and follow-up samples for HBsAg, total anti-HBc, qualitative and quantitative anti-HBs (Monolisa, Bio-Rad Laboratories), IgM anti-HBc (ARCHITECT, Abbott), and alanine aminotransferase (Quest Diagnostics). Donors whose HBV DNA reactivity could be confirmed in index samples or who had serologic evidence of HBV infection in follow-up samples were considered to have HBV infection. The sexual partner of each of these donors was contacted for follow-up, if possible. Screening of a subgroup of donors for anti-HBs was performed as an indicator of HBV vaccine penetrance in the donor population (for details, see the Supplementary Appendix, available with the full text of this article at NEJM.org). Molecular analyses of donor and partner samples were performed in the Geissen and Cambridge laboratories (see the Supplementary Appendix, available with the full text of this article at NEJM.org).

HIV AND HCV RNA CONFIRMATION

Confirmation of the presence of HIV and HCV RNA included HIV and HCV qualitative and quantitative PCR and HCV genotyping performed at the National Genetics Institute. Antibodies against HIV types 1 and 2 (HIV-1/HIV-2 rDNA EIA, Abbott Laboratories) and HCV (HCV 3.0 ELISA, Ortho Clinical Diagnostics) were used for serologic testing of index and follow-up samples. Serologic confirmation of reactive samples was performed with tests licensed by the Food and Drug Administration.[18] Sexual partners were contacted if possible, as described for HBV confirmation.

STUDY OVERSIGHT

The study was funded in part by Gen-Probe and Novartis Vaccines and Diagnostics, which provided the Ultrio test kits and TIGRIS instruments. The American Red Cross provided a clinical-trial site in support of an investigational protocol designed to determine the frequency of donors who test HBV-seronegative but DNA-positive so that an HBV DNA blood-donation-screening claim could be justified for the Ultrio assay performed on the TIGRIS automated platform. The study protocol was designed and executed by the American Red Cross and its collaborators.

Table 1. Confirmed Results on Nucleic Acid Testing and Serologic Analysis of 3,694,858 Blood Donations.*

Classification	HBV		HIV		HCV	
	no.	*frequency*	*no.*	*frequency*	*no.*	*frequency*
Positive on nucleic acid testing and serologic analysis	426†‡	1 in 8673	231	1 in 15,995	1426	1 in 2591
Positive on nucleic acid testing only	9	1 in 410,540	2	1 in 1,847,429	15	1 in 246,324
Positive on serologic analysis only	178†§	1 in 20,758	29	1 in 127,409	517	1 in 7147

* HBV denotes hepatitis B virus, HCV hepatitis C virus, and HIV human immunodeficiency virus.
† Positive results for HBV on serologic analysis indicate the presence of hepatitis B surface antigen (HBsAg).
‡ Of the 426 donations that were positive for HBV DNA and HBsAg (by neutralization), 407 (96%) were reactive to antibodies against hepatitis B core antigen (anti-HBc).
§ Of the 178 donations that were negative for HBV DNA and positive for HBsAg (by neutralization), 86 (48%) were reactive to anti-HBc.

Table 2. Characteristics of 15 Donors with HCV Infection and 2 with HIV Infection.*

Donor No.	Sex	Donor Status	Follow-up	Age	HCV RNA	Genotype
			days	*yr*	*copies/ml*	
HCV						
013–1	M	Repeat		55	<100	NA
Follow-up			11		3,400,000	3A
Partner	F			NA	3,800,000	3A
003–1	M	Repeat		63	12,000	2
011–1	F	First time		24	240,000	3A
012–1	M	Repeat		77	380,000	1A
074–1	M	First time		34	4,900,000	1A
074–2	F	First time		24	6,600,000	1A
074–3	M	First time		17	8,400,000	2B
011–2	M	First time		38	11,000,000	3A
012–2	M	Repeat		49	19,000,000	1B
Follow-up			18		370,000,000	1B
011–3	F	Repeat		24	21,000,000	3A
074–4	M	First time		21	31,000,000	1A
055–1	F	Repeat		48	33,000,000	3A
012–3	M	Repeat		52	41,000,000	3A
054–1	M	Repeat		45	43,000,000	2
019–1	F	First time		30	53,000,000	1A
HIV						
074–5	M	Repeat		16	44,000	NA
074–6	M	Repeat		29	340,000	NA

* Donors with hepatitis C virus (HCV) or human immunodeficiency virus (HIV) are identified by a four-number designation. The first three numbers indicate the collection site, and the number after the dash indicates the donor-sequence number at the given site. Thus, at site 074, six seronegative, RNA-positive donors were identified. NA denotes not available.

RESULTS

DETECTION OF HBV

In 2008, a total of 3,694,858 donations from 2,137,275 donors were screened with the use of Ultrio. Of these samples, 576,940 donations (16%) were screened individually by nucleic acid testing and the remainder were screened in minipools. After virus-specific discriminatory testing, 9 HBV-seronegative donors were confirmed as having HBV infection by additional testing (1 of 410,540 donations) (Table 1). Of the 9 infected donors, 7 (of whom 6 had been vaccinated) consented to participate in the follow-up study, as did the sexual partners of 4 donors. The remaining 2 donors were confirmed as having HBV infection at their index donation from the retrieved plasma unit by repeat nucleic acid testing. All but 1 of the 9 donors with HBV infection were identified by means of minipool nucleic acid testing; the remaining donor (019) required individual-donation nucleic acid testing for detection and tested nonreactive by intermediate dilutions. Of the 9 donors with HBV infection, 8 were repeat donors, and 1 was a first-time donor (055). The mean age of the infected donors was 26 years; 6 of the donors were men.

DETECTION OF HCV AND HIV

Fifteen HCV-seronegative donors had confirmed positive results for HCV RNA on PCR assay at their index donation. Repeat nucleic acid testing of the retrieved plasma unit further confirmed the index results, including identical genotypes and similar viral loads. The 15 HCV-infected donors are shown in order of increasing viral load at the

Table 3. Characteristics of Nine Blood Donors with Hepatitis B Virus (HBV) Infection in Index and Follow-up Samples and in Samples from Sexual Partners.*

Donor No.	Sex	Age	Receipt of HBV Vaccine (yr)	HBV DNA at Index Donation	Duration of Detected Viremia	Anti-HBs Level at Index Donation
		yr		*IU/ml*	*days*	*IU/liter*
001	M	22	Yes (1992)	11	45 to 69	Negative¶
Partner	F	23	No	1.9×10^8	NA	Negative
003	F	37	Yes (1981)	86	44 to 70	3 (ruled nonimmune)
Partner	M	38	No	3.6×10^8	NA	Negative
011	F	17	Yes (1998)	13	137 to 168	11
Partner	M	19	No	2.6×10^8	NA	Negative
013	M	27	Yes (2001)	35	34 to 42	43
Partner	F	25	No	1.8×10^6	NA	Negative
029**	M	19	Yes (unknown)††	30	NA	96
042	M	28	Yes (unknown)††	27	72 to 107	33
019**	F	44	No	21	NA	Negative
055	M	20	No	47	70 to >125	Negative
074	F	24	No	18	73 to 115	Negative

* Donors are identified by a three-number designation, indicating the collection site. At each site, there was only one seronegative, DNA-positive donor. ALT denotes alanine aminotransferase, anti-HBc antibodies against hepatitis B core antigen, anti-HBs antibodies against hepatitis B surface antigen, HBsAg hepatitis B surface antigen, and NA not available.
† The number preceding the genotype, or subgenotype in donors 029 and 042, indicates the number of clones of this genotype or subgenotype in the entire quasispecies.
‡ A value higher than 30 U per liter was considered to be elevated.
§ "Cons" indicates consensus sequence obtained by direct sequencing. The number preceding "x" indicates the number of clones carrying the substitution; "s" indicates the location of the amino acid substitution in the HBsAg gene.
¶ The donor became positive for anti-HBs (100 IU per liter) within 45 days, which was up to 24 days before seroconversion to IgM anti-HBc.
‖ The donor was reactive at the index donation.
** The donor declined enrollment in the follow-up study.
†† The donor could not recall the year of vaccination.

index donation (median, 11 million copies per milliliter) in Table 2. The rate of HCV RNA–positive, seronegative donations was 1 in 246,324 (Table 1). Of the 15 donors, there was a nearly even split between first-time and repeat donors, with a mean age of 40 years; 10 of the donors were men. The female sexual partner of donor 013-1 was available for testing; the two had the same 3A genotype, implying infection from a common source or through sexual transmission.

Two donors had confirmed positive results for HIV RNA on PCR assay at their index donation, with viral loads of 44,000 and 340,000 copies per milliliter (Table 2). Testing of the plasma unit confirmed the index test results. These two donors, who were both seronegative, were male repeat donors who were 16 and 29 years of age. The HIV RNA–positive donation rate was 1 in 1,847,429 donations (Table 1).

In a combined analysis of study findings, among 75 samples from seronegative donors that were reactive on Ultrio and discriminatory testing, there were 26 confirmed infections (9 HBV, 15 HCV, and 2 HIV), or 1 in 153,952 donations. Thus, the positive predictive value for the Ultrio triplex assay in seronegative samples, in-

Days to Seroconversion			Genotype or Subgenotype†	ALT‡	Number of Clones Sequenced	Amino Acid Substitutions on S Protein§
HBsAg	Anti-HBc (IgM and total)	Anti-HBs				
	days			*U/liter*	*no.*	
No	69	45	C2	7 to 16	20	20xsG145R, sV184G, W199 stop
NA	NA	NA	C2	31	14	Cons sG145R, 3xsG145R, sV184G
No	70	20	A2	3 to 8	11	G102D, T134A
NA	NA	NA	A2	17	10	
108	168	Reactive‖	F1	4 to 7	12	Wild type
NA	NA	NA	F1	36	11	4xsY72 stop
No	No	Reactive‖	B2	8 to 19	10	1xsG112R, 1xsA128T
NA	NA	NA	B2	38	9	Wild type
NA	NA	Reactive‖	7D, 4A2, 1A2/D	9	10	2xsQ54 frameshift
72	107	Reactive‖	6D, 2A2	3 to 12	8	sY134H, sT143A
NA	NA	NA	A2	18	15	sG112R, sT118 M, sF134L
40	70	125	A2	9 to 640	18	C124Y, sD151L, sW156R
No	73	115	A2	9 to 119	15	sT126A

cluding virus-specific discrimination, was 35%, which is similar to the predictive value of other screening assays. Two of the 26 samples required individual-donation nucleic acid testing for detection, for a yield of 1 in 288,470 donations. The rates of detection on serologic analysis were higher than the rates on nucleic acid testing for all three viruses, underscoring the need for both nucleic acid testing and serologic screening (Table 1).

COURSE OF HBV INFECTION IN INDEX DONORS

The HBV viral load was consistently low in all index samples from the nine infected donors (11 to 86 IU per milliliter) (Table 3). Five index samples contained only HBV DNA, with no serologic HBV markers, and four samples contained low-level anti-HBs (3 to 96 IU per liter), all from donors who had been vaccinated against HBV. Attempts were made to contact all nine donors. Two donors (019 and 029) did not respond; the other seven donors had viremia for 34 to at least 137 days but subsequently had undetectable levels of HBV DNA (Table 3). Samples from these seven donors were obtained regularly for 119 to 320 days. IgM anti-HBc (confirming acute infection) developed in all but one donor (013); this donor had the shortest period of viremia and did not have detectable HBsAg or total anti-HBc. One unvaccinated donor (055) had the classic pattern of window-period evolution accompanied by clinical signs of disease, with the presence of HBsAg from 40 to at least 70 days, corresponding to a peak viral load of 1.6×10^8 IU per milliliter and an alanine aminotransferase level of 640 U per liter. IgM and total anti-HBc appeared on day 70, and anti-HBs appeared on day 125 (Table 3 and Fig. 1A). Another unvaccinated donor (074) who did not have anti-HBs at baseline became reactive to IgM and total anti-HBc on day 73, with seroconversion to anti-HBs on day 115. Although HBsAg was never detected, there were clinical signs of disease, on the basis of an elevated alanine aminotransferase level (119 U per liter) on day 73.

Follow-up studies were performed for five of the six donors with a history of HBV vaccination;

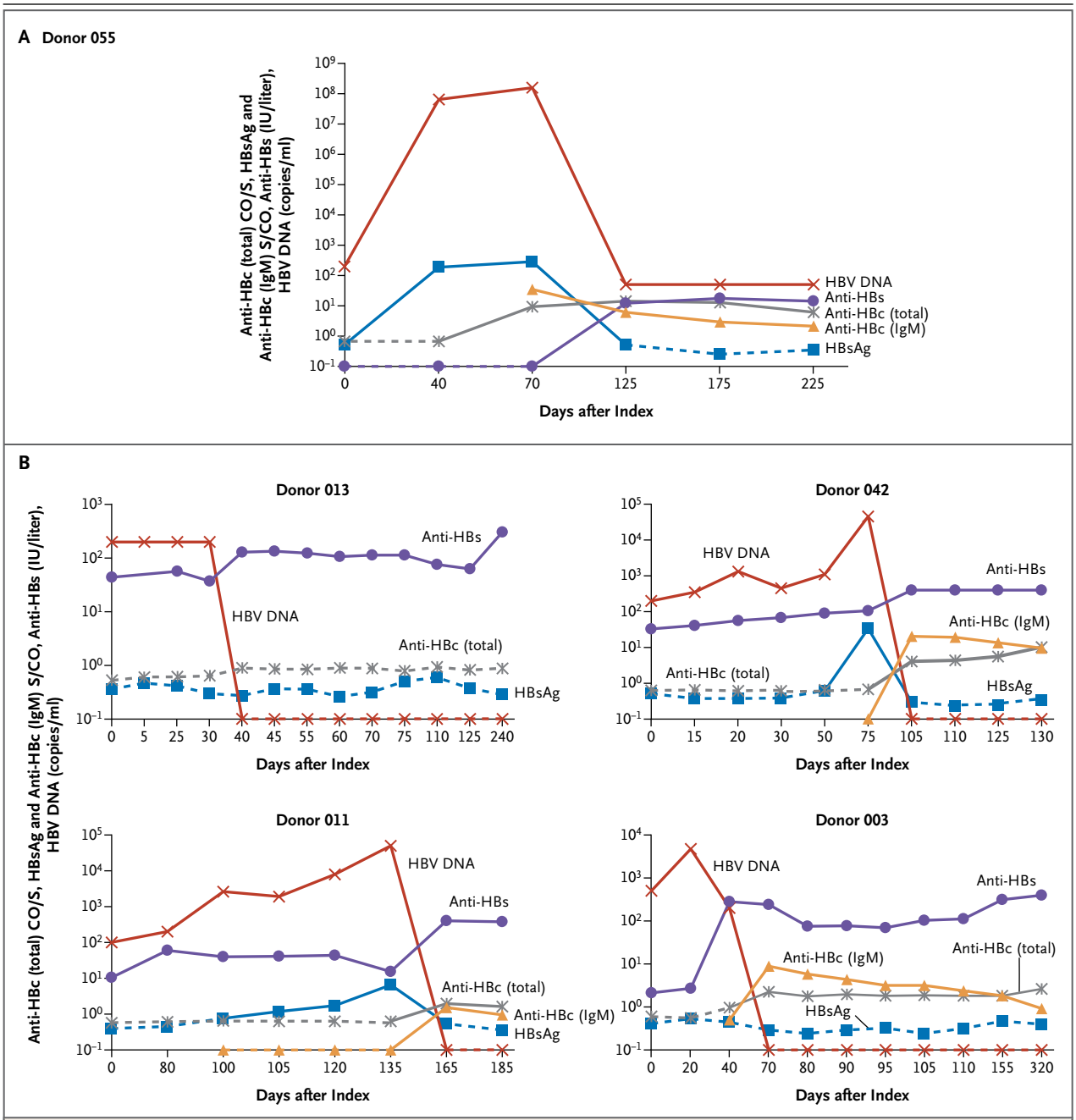

Figure 1. Evolution of Serologic Markers for Hepatitis B Virus (HBV) after Index Donation from Five Donors with Acute HBV Infection.

The results of follow-up analyses of virologic and serologic markers for HBV are shown for one donor who did not receive the HBV vaccine (Panel A) and for four vaccinated donors (Panel B). Included in the analyses are values for HBV DNA (in copies per milliliter). Values for hepatitis B surface antigen (HBsAg) and for IgM antibodies against hepatitis B core antigen (anti-HBc) are reported as signal-to-cutoff (S/CO) ratios, where values of 1.00 or more are considered to indicate reactivity. Values for anti-HBc (total) are reported as the reciprocal of S/CO since the assay format is based on competitive inhibition. Values of 10 or more for antibodies against hepatitis B surface antigen (anti-HBs) are considered to indicate immunity. Dashed lines indicate values below the threshold of detection.

four of the five were positive for anti-HBs and for HBV DNA at baseline. Transiently low levels of HBsAg were observed in two of these donors (011 and 042), whereas anti-HBs remained reactive (Table 3 and Fig. 1B). In these two donors, an increase in anti-HBs corresponded to the disappearance of HBsAg. IgM and total anti-HBc became positive on day 107 in one donor (042) and on day 168 in the other (011). Donor 013 did not have detectable HBsAg or anti-HBc for 9 months, but an increase in anti-HBs occurred on day 42, when HBV DNA became undetectable (Table 3 and Fig. 1B). The fourth donor (003) had received a plasma-derived HBV vaccine 27 years earlier; her level of anti-HBs in the index donation (3 IU per liter) was considered to be nonimmune. HBV DNA remained detectable for up to 70 days, at which time IgM and total anti-HBc appeared. HBsAg was never detected; however, an anamnestic response for anti-HBs was observed in the first follow-up sample (obtained on day 20). A fifth vaccinated donor (001) was negative for anti-HBs at baseline but had seroconversion (100 IU per liter) within 45 days, a finding that was consistent with an anamnestic response, since it preceded the detection of total and IgM anti-HBc by up to 24 days. Despite the detection of IgM anti-HBc in four of the five vaccinated donors, no increased levels of alanine aminotransferase were noted, which further substantiated the inconsequential nature of these infections.

HBV genotypes or subgenotypes of the 6 vaccinated donors were C2 (in donor 001), A2 (in donor 003), F1 (in donor 011), B2 (in donor 013), and sequences representing both D and A2 (in donors 029 and 042), as compared with A2 in all unvaccinated donors ($P=0.048$ by Fisher's exact test, two-tailed, for the comparison between the dominant strains in the 6 vaccinated donors and in the 3 unvaccinated donors) (Table 3 and Fig. 2, and the Supplementary Appendix). Figure 3 shows the levels of anti-HBs in 520 randomly selected donors who were not infected with HBV. Of the 231 immune donors in this subgroup (44%), more than 65% were 29 years of age or younger, which was consistent with the mean age of 26 years for the 9 donors with HBV infection, of whom 6 had also been vaccinated.

ORIGIN OF HBV INFECTION

The sexual partners of infected donors were traced when possible, and four were found to have HBV infection. In all cases, the sequences of the presurface and surface (pre-S/S) regions of the HBsAg gene were nearly identical at the nucleotide level (<0.1% divergence) and the amino acid level in two laboratories, as were the full genome sequences in donor 013 and his sexual partner, which strongly suggests that infection occurred between vaccinated donors and their sexual partners who carried high viral loads ($>1.0\times10^6$ IU per milliliter) (Table 3). All these partners were highly positive for HBsAg and anti-HBc but were negative for anti-HBs and IgM anti-HBc, indicating chronic carriage. Three of the four partners had mild alanine aminotransferase elevations (range, 31 to 38 U per liter), which were consistent with chronic hepatitis B. The transfer of a mutated minority variant to one donor (001) from his partner further confirms sexual transmission and immune selection. The fifth and sixth vaccinated donors were health care workers whose acute infections were probably the result of occupational exposure.

DISCUSSION

Analysis of the results of blood-donor testing, along with follow-up analyses of infected donors, permits assessment of the details of early infection and modeling of the residual risk of infectivity from transfusion.[13,18] In this study, we found some unexpected patterns of early HBV infection as a result of evaluating HBV nucleic acid testing among 3.7 million blood donations. On the basis of modeling studies, we anticipated two to four seronegative, HBV DNA–positive samples from minipool nucleic acid testing and one seronegative, HBV DNA–positive sample from single-donation testing.[14] In fact, we found nine seronegative, HBV DNA–positive samples, and all but one were detected on minipool nucleic acid testing, for a total rate of 1 per 410,540 donations. Three of the infected donors appeared to have conventional window-period infection, which was consistent with expected findings. Unexpectedly, five of the other six infected donors had low-level anti-HBs (3 to 96 IU per liter) or a rapid anamnestic response, attributable to the receipt of HBV vaccine 7 to 27 years earlier. The five vaccinated donors had a brief, transient course of infection with no evidence of disease and very low or absent expression of HBsAg. Viremia, which was of short duration, ranged from 11 to 86 IU per milli-

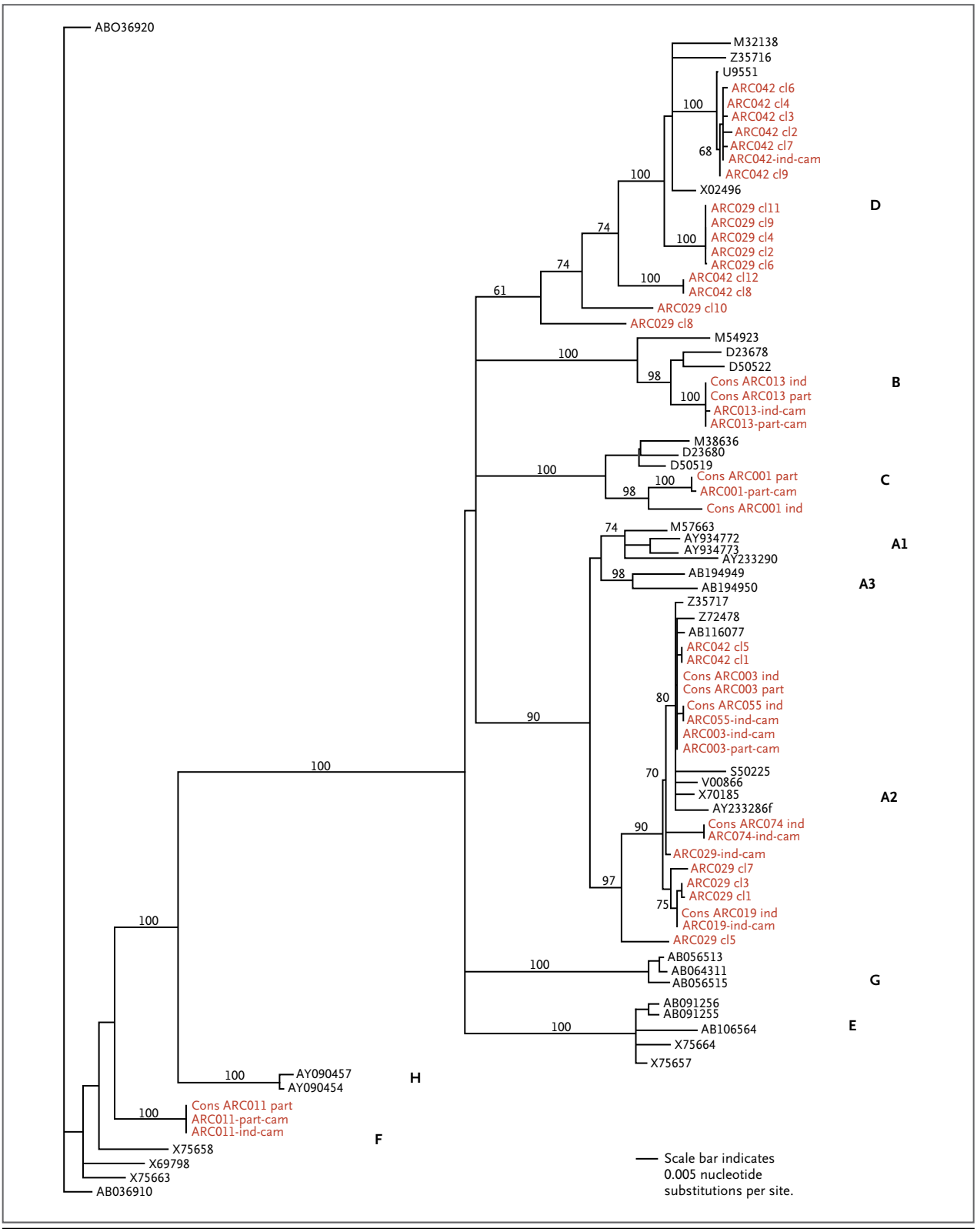

Figure 2 (facing page). Phylogenetic Analysis of Nine Index Blood Donors and Their Sexual Partners in a 1200-Nucleotide Region, Including the Pre-S/S Gene.

Individual strains that are included in the study are shown in red, and genotype references from GenBank are shown in black. Study samples are coded as ARC (indicating the American Red Cross), followed by a three-digit code number. The abbreviation "ind" indicates an index sample, and "part" indicates a sample from a sexual partner of an index donor. Sample codes ending with "cam" indicate consensus sequences obtained in the Cambridge laboratory. Sample codes preceded by "cons" indicate consensus of all clones obtained at the Giessen laboratory for a particular strain. For the two samples that were identified as being coinfected with two different genotypes, "cl" plus a number indicates the clone number within a sample quasispecies. In all cases, consensus sequences that were obtained either in Cambridge or in Giessen were identical or differed by fewer than three nucleotides. The letters to the right of the strains indicate the HBV genotype (B through H) or subgenotype (A1, A2, and A3). The numbers to the left of the strains indicate genomic distance calculations (bootstrap values in percentages), in which values of more than 70% are considered to be significantly different.

liter at donation. Four of the five vaccinated donors who were followed were also positive for IgM anti-HBc, confirming acute infection. Smaller studies in the United States have typically shown approximately one HBV-infected donor per 600,000 donations screened.[15] In countries where HBV infection is endemic, rates are much higher.[10,19]

The three acute infections among nonvaccinated donors were of subgenotype A2, which is the most frequent genotype in the United States and is the parent strain of the HBV vaccine. Elevations in alanine aminotransferase levels developed in two of these donors (119 and 640 U per liter) during follow-up. In contrast, only one of the six previously vaccinated donors was uniquely infected with the A2 strain; she had received the plasma-derived vaccine 27 years earlier. All other donors were infected exclusively or predominantly with non-A2 strains. None of the vaccinated donors had any signs of elevated levels of alanine aminotransferase. Overall, the data emphasize the protective effect of the vaccine, since the observed infections were transient, blunted, and without effect on liver function. Our findings thus suggest that the vaccine may be less effective for non-A2 infections. Of interest, four donors with acute HBV infection were discovered to have sexual partners who were chronic HBV carriers with high viral loads of wild-type (or in one case, mutated) HBV DNA. These infected donors had the same subgenotype and full genome sequences compatible with transmission.

The blood of donors with acute HBV DNA–positive infection during the window period is likely to be highly infectious in transfusion recipients.[11,12] The significance of infection in vaccinated donors is less clear. In one study, blood donations that were positive for HBV DNA with detectable levels of anti-HBs were not infectious in any of 22 recipients, as compared with a rate of infection of 27% among 37 recipients of blood that was devoid of anti-HBs.[11] Similarly, the absence of infectivity in the presence of anti-HBs has been observed in other studies.[2,20-23] Conversely, blood containing HBV DNA with low-level anti-HBs (<75 IU per liter) may carry a risk of transmission leading to acute hepatitis.[24] These study findings may be relevant to decisions about the need to implement screening for HBV DNA among blood donors.

The rates of RNA-positive HIV and HCV infection among seronegative donations in our study (1 in 1.85 million for HIV and 1 in 246,000 for HCV) parallel those observed by the American Red Cross among more than 66 million donations tested in the 10 years since the introduction of nucleic acid testing for HIV and HCV (32 HIV-positive donations, or 1 in 2 million, and 244 HCV-positive donations, or 1 in 270,000)[18] and are consistent with earlier reports.[25] The characteristics of the 15 donors with HCV infection and the 2 donors with HIV infection in our study were similar to those reported previously.[18]

The infrequent detection of transfusion-transmitted infection contributes to the low cost-effectiveness of nucleic acid testing (for details, see the Supplementary Appendix).[26,27] During 10 years of nucleic acid testing for HIV and HCV in the United States, five HIV-infected donors have transmitted the virus to six of eight living transfusion recipients. Two recipients were infected by one donor, and two other window-period donors each infected one recipient. In two cases, only one of two living recipients became infected; of these last two cases of differential transmission (i.e., in which one recipient became infected and the other did not), one case is unpublished.[28-31] In contrast, there has been

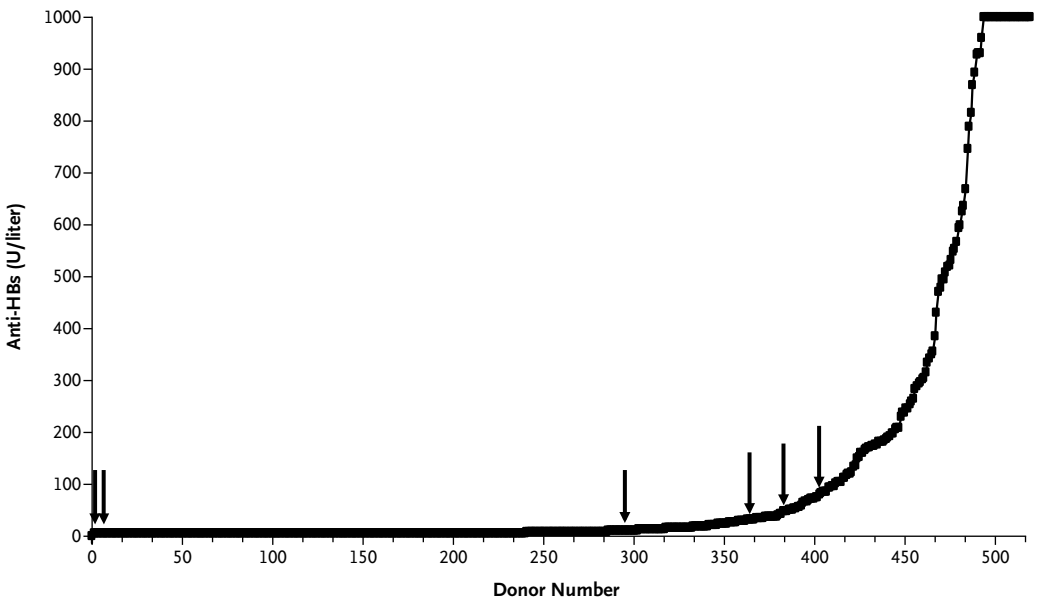

Figure 3. Levels of Antibodies against Hepatitis B Surface Antigen (Anti-HBs) in 520 Randomly Selected Samples from U.S. Blood Donors.

Shown are results for 520 blood donors who tested negative for hepatitis B virus (HBV) DNA, hepatitis B surface antigen (HBsAg), and antibodies against hepatitis B core antigen (anti-HBc), plotted in order of increasing level of anti-HBs antibodies. Each symbol represents an individual donor; 44% of donors were considered to have immunity against HBV (i.e., an anti-HBs value of 10 IU per liter or more). The arrows indicate anti-HBs levels that correspond to values for the six HBV-vaccinated study donors who were found to be positive for HBV DNA: negative, 3, 11, 33, 43, and 96 IU per liter.

only one report of suspected transmission of HCV through transfusion.[32] For HBV, there has been no documentation of transmission through transfusion in the United States since the introduction of ultrasensitive screening for HBsAg in 2006. The two cases of differential transmission of HIV that were observed by the American Red Cross, in which a high-volume plasma component transmitted the virus but packed red cells containing low amounts of plasma did not, indicate that the sensitivity of nucleic acid testing, even in minipools, may approach the threshold for infection. In any event, since the implementation of nucleic acid testing and sensitive serologic assays, viral transmission by transfusion from donors who previously had negative results on both serologic and nucleic acid testing is a rare finding[27] (for details, see the Supplementary Appendix).

In summary, this study showed a higher-than-expected rate of HBV infection with the use of triplex nucleic acid testing, mainly in donors who had been vaccinated against HBV and who would not have been identified by routine screening for HBsAg or anti-HBc. However, these acute HBV infections rapidly resolved and are of inconsequential clinical significance, but their potential for transmission remains unresolved. Our findings show the efficacy of the HBV vaccine for the prevention of clinical disease but not infection, and the cost of interdicting donations that contain HBV DNA from seronegative donors is high in the face of unknown benefit. Because of the high cost of introducing new screening assays for blood donors and the inability to document cost-effectiveness similar to that of other medical interventions, the continued development of new tests either to replace older tests or to detect newly identified agents[33] is in question.

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