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一般的名称			研究報告の公表状況	Correlates of hepatitis C virus (HCV) RNA negativity among HCV-seropositive blood donors Busch, M.P. et al, Transfusion. 46, 469 - 475 (2006)	公表国 米国	
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研究報告の概要	使用上の注意記載状況・その他参考事項等
C 型肝炎ウイルス (以下, HCV) 感染した約 20%の人はウイルス血症が消滅するが, 理由は明確ではない。一方, HCV 初感染者の大多数は無症候であるが, 数十年後に臨床症状を発症する。すなわちこれは, 供血者の中に臨床上の潜在 HCV 感染者がいるということである。 本研究では, 1999 年～2001 年 12 月に NAT を実施した合衆国の 5 つの血液センターで集められた 257 万 9290 名の同種供血者における HCV 抗体と核酸増幅検査 (以下, NAT) データ, 肝酵素アラニン・アミノトランスフェラーゼ (ALT) を調査すると同時に人口学的特性 (年齢, 性別, 人種及び教育レベル) も調査した。 NAT による HCV RNA 陰性患者は, セロコンバージョンまでの期間にかかわらず, 初回供血者 (19.6%) より反復供血者 (55.9%) の方が多かった。初回供血の HCV RNA 陰性者と RNA 陽性者の ALT レベルを比較すると, ALT が 60IU/L 未満の患者の比率はそれぞれ 98.51%, 83.78%, ALT が 120IU/L 以上ではそれぞれ 0.75%, 13.38%であり, ALT レベルは初回供血の HCV RNA 陰性者の方がより正常に近い傾向を示した。 また, 初回供血者の HCV RNA 陰性の比率は, アジア系, 非ヒスパニック黒人系よりも (非ヒスパニック) 白人系で有意に高かった。 さらに有意ではないが, 男性より女性のほうが NAT 陰性の比率が高かった。 なお, この調査は, HCV 罹患率・有病率の高い合衆国南東部を除いた血液センターで実施されており, NAT は個々の供血者ではなくミニプールに対して行われた。	BYL-2006-0214

報告企業の意見	今後の対応
弊社の血漿分画製剤においても, 1988 年よりミニプール及びプール血漿での HCV に対する NAT を実施しているが, 無症候セロコンバージョンが起きた供血者の血漿が使用される可能性は否定できない。しかしながら, 製造工程におけるウイルス除去工程により, 血漿分画製剤の HCV に関する安全性は確保されていると考えられる。	現時点で新たな安全対策上の措置を講じる必要は無いと考える。引き続き関連情報の収集に努める。

TRANSFUSION COMPLICATIONS

Correlates of hepatitis C virus (HCV) RNA negativity among HCV-seropositive blood donors

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BACKGROUND: Approximately 20 percent of persons infected with hepatitis C virus (HCV) clear viremia. Factors associated with resolution of viremia are not well defined. Implementation of routine nucleic acid testing (NAT) of blood donors has yielded a large data set for analysis of demographic correlates of resolved viremia.

STUDY DESIGN AND METHODS: HCV antibody and NAT data, liver enzyme (alanine aminotransferase [ALT]) results, and donor demographic characteristics were compiled for 2,579,290 allogeneic donations given at five large blood centers after NAT implementation in 1999 through December 2001. Donation HCV RNA status was compared between first-time donors categorized by ALT levels, sex, age, race and/or ethnicity, country of birth, level of education, blood center location, and blood group, with chi-square tests and multivariable logistic regression methods.

RESULTS: Of 35 confirmed-seropositive repeat donors, 19 (54.3%) tested negative for the presence of HCV RNA; there was no association between RNA status and preseroconversion intervals ($p = 0.74$). Of 2105 RIBA-positive, first-time donors, 402 (19.1%) tested negative for the presence of HCV RNA by NAT (presumptive resolved infections). There were significant differences in the frequency of RNA negativity among first-time donors categorized by ALT levels and by race and/or ethnicity. ALT levels were more likely to be elevated in RNA-positive, first-time donors ($p < 0.0001$). Viremia was less likely to resolve in Asian (8.2%) and black non-Hispanic (14.4%) donors than in white non-Hispanic (20.7%), Hispanic (22.1%), and other race and/or ethnicity (22.1%) donors ($p = 0.02$). No significant associations were found for age, sex, country of origin, level of education, blood type, and donor center location.

CONCLUSION: These results confirm that the frequency of HCV RNA negativity among seropositive persons differs by race and/or ethnicity. Follow-up studies of donors with resolved viremia are warranted to further elucidate viral, immunologic, and genetic factors underlying spontaneous viral clearance.

Only about 15 percent of persons recently infected with hepatitis C virus (HCV) develop a clinically overt hepatitis syndrome, which is generally mild, occurs within 5 to 12 weeks of exposure (mean, 8 weeks), and lasts from 2 to 12 weeks.[1,2] Although 85 percent of primary HCV infections are asymptomatic, most studies indicate that 70 to 80 percent of these infections become chronic with asymptomatic viremia generally persisting for decades before later disease manifestations.[1,3] This results in a reservoir of clinically occult HCV infections in persons presenting to donate blood.[3,4] HCV seroprevalence (confirmed by

ABBREVIATIONS: ARC = American Red Cross; BCP = Blood Centers of the Pacific; IDU(s) = injection drug use(rs); MP(s) = minipool(s); REDS = Retrovirus Epidemiology Donor Study; TMA = transcription-mediated amplification.

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recombinant immunoblot assay [RIBA]) among first-time donors in the United States ranges from 0.2 to 0.3 percent (reflecting a steady decrease from 0.6% in 1992 owing to increasingly effective education and deferral procedures). With approximately 2.6 million first-time donations per year in the United States, we estimated that 6000 to 9000 HCV-seropositive infections are diagnosed annually as a result of donor screening (data obtained from the American Red Cross [ARC] national database). Since introduction of nucleic acid amplification testing (NAT) in 1999, the viremia status of seropositive donors is routinely available. In addition, NAT screening detects approximately 60 additional HCV-infected donors in the acute viremic, seronegative phase of infection each year (data obtained from the ARC national database).[5,6] Results from interview studies of seropositive US blood donors have demonstrated that injection drug use (IDU) many years before the HCV-positive donation is the likely source of infection for the majority of cases, with transfusions before implementation of HCV screening and other parenteral and sexual risk factors implicated to a lesser extent.[7,8]

Evidence from human and chimpanzee studies indicates that long-term viremia status (i.e., clearance vs. persistence) is generally determined within 6 months but occasionally as long as 12 to 24 months after infection.[1,9-11] The mechanisms and determinants of apparent viral clearance after seroconversion (as demonstrated by RNA negativity but HCV seropositivity) are not well understood but likely involve a combination of host and viral factors, including mode of acquisition; dose and quasispecies complexity of HCV in the source inoculum; viral genotype and/or subtype; patient race and/or ethnicity, HLA type, sex, and age at infection; and numerous host immune response variables.[12-21] Of note, symptomatic acute infections have been associated with an increased rate of viral clearance (up to 50%) compared to asymptomatic infections, probably reflecting the dual effect of a more robust immune response that eradicates infected hepatocytes and results in manifestations of clinical hepatitis.[1,2]

Owing to the large numbers, diversity, and low-risk characteristics of HCV-infected blood donors, this population may offer unique insights into correlates of HCV resolution, relative to those from studies of patients with symptomatic acute infections, historically identified transfusion recipients, or prospectively followed high-risk populations (primarily active IDUs).[1,3] To investigate laboratory and demographic correlates of HCV RNA negativity in HCV-seropositive blood donors, we compiled data on first-time allogeneic (whole-blood community, directed, and apheresis) donations collected at five blood centers participating in the National Heart, Lung, and Blood Institute Retrovirus Epidemiology Donor Study (REDS) that tested HCV-seropositive between the start of HCV RNA NAT in mid-1999 through December 31, 2001. We also evaluated the NAT status of repeat allogeneic donors who gave an HCV antibody–positive donation in the NAT period.

MATERIALS AND METHODS

Five blood centers collecting approximately 8 percent of all US allogeneic donations participated in REDS including three ARC Blood Services regions (Greater Chesapeake and Potomac Region [Baltimore, MD, and Washington, DC], Southeastern Michigan Region [Detroit, MI], and Southern California Region [Los Angeles, CA]); the Blood Centers of the Pacific (BCP, San Francisco, CA); and the Sylvan N. Goldman Oklahoma Blood Institute ([Oklahoma City, OK]). Information on donation type, donation date, demographic characteristics (age, sex, race and/or ethnicity, education level, country of birth), first-time and repeat donor status, and screening and confirmatory test results have been compiled in a database by the REDS coordinating center (Westat, Inc., Rockville, MD) since 1991. The REDS protocol was approved by the institutional review board at each center.

We identified first-time allogeneic (whole-blood community or directed and apheresis) donations that were confirmed HCV antibody–positive and were collected between April 1999, the approximate start of HCV NAT screening at REDS centers, and December 31, 2001 (the study period). The data set for the three ARC centers was subsequently limited to HCV-seropositive donations given subsequent to September 8, 1999, because before that date seroreactive donations were frequently identified before pooling and consequently not subjected to NAT screening. During the study period, screening for antibodies to HCV was conducted with a third generation enzyme-linked immunosorbent assay (Ortho HCV Version 3.0 ELISA test system, Ortho-Clinical Diagnostics, Inc., Raritan NJ). A third-generation (Chiron RIBA HCV 3.0 strip immunoblot assay, Chiron Corp., Emeryville, CA) was used as the confirmatory test by all centers, except for one center (BCP), which used either second-generation RIBA HCV or third-generation HCV assay versions between April and July 1999, before switching exclusively to the third-generation version on July 15, 1999. NAT was conducted with a human immunodeficiency virus-1 (HIV-1) and HCV transcription-mediated amplification (TMA) system (Procleix, Gen-Probe, Inc., San Diego, CA) at four centers (ARC centers and BCP) and a second-generation HCV screening test (COBAS AmpliScreen, Roche Molecular Systems, Inc., Pleasanton CA) at one center (Oklahoma Blood Institute).[6,22] For the Procleix TMA system, minipools (MPs) consisting of 16 individual donations were screened by the multiplex HIV-1 and HCV assay (except for BCP where donations were screened with MPs of 24 for the initial 14 months of NAT screening).[6,22,23] Once a reactive MP was identified, the corresponding individual donations were tested by the multiplex HIV-1 and HCV

assay, followed, if reactive, by discriminatory HCV and HIV-1 TMA assays to identify the viremic donation. For this analysis, only those specimens that tested reactive by the discriminatory HCV TMA were categorized as HCV RNA–positive. The COBAS AmpliScreen HCV assay was performed on MPs of 24 donations, with two-step resolution with six-member intermediate pools, followed by individual donation testing to identify the HCV RNA–positive donation(s). Both the Procleix and the AmpliScreen systems are highly sensitive with 50 percent detection limits of 12 HCV copies per mL on neat samples based on probit analyses and projected sensitivities of MP NAT screening of fewer than 250 copies per mL.[22,23]

We also identified repeat allogeneic donors who had given at least one third-generation HCV ELISA–nonreactive donation since January through June 1996 (time of third-generation HCV ELISA implementation) followed by an HCV ELISA–reactive and third-generation RIBA–positive donation in the NAT study period (April-September 1999, to December 31, 2001). We evaluated the proportions of third-generation RIBA–positive donations that were RNA-positive or -negative as a function of estimated time since infection by evaluating NAT status as a function of the length of the seroconversion interval or time between the HCV antibody–negative and HCV antibody–positive donations.

To evaluate if HCV RNA negativity in seropositive donors was associated with demographic characteristics, we evaluated the number of HCV RNA-negative (presumptive resolved infections) and RNA-positive (presumptive chronic infections) donations from first-time seropositive donors in various demographic groups and used chi-square tests to compare the proportions of HCV RNA-negative donations between demographic groups. The demographic characteristics evaluated included age (<25, 25-34, 35-44, 45-54, 55-65, and ≥65 years old), sex, race and/or ethnicity (white non-Hispanic, black non-Hispanic, Hispanic, Asian, other non-Hispanic), country of birth (US; non-US), education level (less than high school degree, high school degree, some college or an associate degree, college degree, graduate or professional degree), and blood center location. We also assessed if the distribution of ABO/Rh blood groups and alanine aminotransferase (ALT) levels (normal, <60 IU/L; low-elevated, 60-120 IU/L; high-elevated, >120 IU/L) differed between the RNA-negative and RNA-positive groups. Missing data were excluded from the analysis (NAT results were not available for 2.4% of donations from HCV antibody–positive, first-time donors; race and/or ethnicity, level of education, and country of birth information were missing for 11.3, 9.3, and 3.8 percent of donations from RIBA-positive first-time donors, respectively; and information on age, sex, blood center, ABO/Rh, and ALT was missing for 0.1% of donations from first-time donors). Logistic regression models were conducted to obtain odds ratios (ORs) that compared the odds of being HCV RNA-negative between demographic groups. We included as independent variables in the adjusted models only those demographic characteristics with a chi-square p value of 0.1. The models were conducted both unadjusted (one demographic characteristic at a time) and adjusted (all demographic variables entered into the model). The adjusted model permitted us to evaluate whether the association between a particular demographic variable and NAT status was confounded by another demographic variable, that is, whether the apparent effect of one demographic variable on NAT nonreactivity could be attributed to the effect of another demographic variable on NAT status.

RESULTS

The five participating blood centers collected 2,579,290 allogeneic donations during the study period (start of routine MP-NAT of seroreactive donations in 1999 through December 31, 2001), including 616,228 first-time (23.9%) and 1,963,062 (76.1%) repeat donations. There were 2105 donations from HCV ELISA–reactive and RIBA-positive first-time donors (seroprevalence, 0.34%); of these 402 (19.6%) tested nonreactive and 1653 (78.5%) reactive by MP-NAT (50 donations [2.4%] from RIBA-positive, first-time donors had missing NAT results and were subsequently excluded from the analysis; Table 1). HCV seroprevalence rates were similar among the five REDS centers; the number of confirmed seropositive donors contributed per center varied from 227 (ARC Southeastern Michigan Region) to 729 (ARC Southern California Region).

We also identified 35 HCV seroconverters who gave at least one HCV ELISA–nonreactive donation subsequent to implementation of the third-generation version of the anti-HCV screening assay in 1996, which was followed by an ELISA-reactive and RIBA-positive donation during the current study period. Of these 35 repeat donors, 19 (54.3%) were HCV MP-NAT–nonreactive on their RIBA-positive donation (MP-NAT was not available for 1 positive donation [2.9%]). The median seroconversion interval length observed for the 34 seroconverters with a NAT result was 22.6 months (range, 3.4 to 60.4 months); 24 of the 34 seroconverters (70.6%) had an interval of at least 1 year. We hypothesized that repeat donors with shorter seroconversion intervals would have higher RNA positivity rates than donors with longer seroconversion intervals, since on average they should have been infected more recently and consequently had less time to clear the HCV virus. As shown in Table 1, however, there was no significant difference in HCV RNA rates in seropositive donors with shorter or longer seroconversion intervals ($p = 0.74$).

Further analyses of correlates of RNA negativity were limited to the 2055 first-time confirmed-seropositive

donors for whom NAT status was available. Significant associations ($p < 0.05$) were observed between HCV RNA negativity, and ALT levels (Table 2) and race and/or ethnicity (Table 3). ALT levels were more likely to be normal in RNA-negative donors than in RNA-positive donors, and high-elevated ALT levels (≥120 IU/L) were much more frequently observed in RNA-positive donors than RNA-negative donors ($p < 0.0001$; Table 2). Asian and black non-Hispanic donors were less likely to be RNA-negative than Hispanic, white non-Hispanic, or other non-Hispanic first-time donors ($p = 0.02$; Table 3). The odds of HCV RNA negativity were significantly lower in Asian donors (unadjusted OR, 0.34; 95% confidence interval [CI], 0.12-0.96) and in black non-Hispanic donors (unadjusted OR, 0.64; 95% CI, 0.44-0.93) than in white non-Hispanic donors (Table 4). These racial and/or ethnic differences were not explained by age or sex differences between race and/or ethnic groups because unadjusted and adjusted (for age and sex) ORs remained similar (Table 4).

Associations between HCV RNA negativity and sex ($p = 0.10$), age ($p = 0.12$), blood center location ($p = 0.17$), country of origin ($p = 0.64$), education ($p = 0.80$), and ABO/Rh status ($p = 0.72$) were all nonsignificant (Table 3).

TABLE 1. Proportion of RIBA-positive donations by first-time and repeat donors who tested HCV RNA–negative by MP-NAT and relationship of preseroconversion intervals for repeat donors by RNA status

Donor status	Number of RIBA-positive donations	HCV RNA–negative*	p Value
First-time	2055	402 (19.6)	
Repeat: length of preseroconversion interval (months)	34	19 (55.9)	
≤6	4	3 (75.0)	
>6-9	2	2 (100.0)	
>9-12	4	2 (50.00)	
>12-24	9	4 (44.4)	
>24-60	15	8 (53.3)	0.74

* Data are reported as number (%). MP-NAT results were not available for 50 RIBA-positive, first-time donors (2.4%) and for 1 RIBA-positive, repeat donor (2.9%).

TABLE 2. Proportion of first-time seropositive donations who were HCV RNA-negative or -positive (by MP-NAT) with normal, low-elevated, or high-elevated ALT levels

	ALT level (IU/L)			
HCV status	Normal (<60)	Low-elevated (60-120)	High-elevated (≥120)	p Value
RNA-negative	396 (98.51)	3 (0.75)	3 (0.75)	
RNA-positive	1384 (83.78)	47 (2.85)	221 (13.38)	<0.0001

* Data are reported as number (%).

DISCUSSION

Investigations in various populations at high risk for HCV infection have identified correlates of HCV RNA clearance, including host factors such as age, sex, and race and/or ethnicity. The effectiveness of the immune response (which is largely determined by host genetics but influenced by age, health status, and previous antigenic exposures) and the corresponding evolution of the virus during the preseroconversion and early postseroconversion phases of infection are probably the major determinants of successful resolution of HCV viremia.[12-21,24-26]

Thomas and coworkers[18] first reported that race and/or ethnicity is significantly associated with clearance of HCV RNA in a study of seropositive IDU in Baltimore, Maryland. These investigators found that 9.3 percent of 729 seropositive black non-Hispanic IDUs resolved HCV infection, compared to 36 percent of 44 nonblack donors. Although our findings confirm a significant association between HCV RNA negativity in seropositive first-time blood donors and race and/or ethnicity, the differences in frequencies between black non-Hispanic blood donors (14.4%) and nonblack donors (e.g., 20.7% for white non-Hispanic donors) are smaller than seen with the Baltimore IDU cohort. This difference may relate to the high rate of HIV coinfection in the IDU cohort (33.4% of HCV-seropositive IDUs were HIV-seropositive, and HIV coinfection was associated with lower rates of HCV clearance) and to the relatively small number of nonblack subjects in that study compared to our study (44 vs. 1153, respectively). We also found that the odds of Asian first-time seropositive donors being RNA-positive was approximately three times that of white non-Hispanic donors. To elucidate the basis for these race and/or ethnicity differences, investigators have begun to correlate HLA and other immune response polymorphisms with HCV clearance status. Strong associations with several HLA class I and II alleles have been detected.[19,20] Persons who have cleared HCV are also more likely than chronic carriers to be homozygous for specific inhibitory killer immunoglobulin-like receptors (KIR) combinations (KIR2DL3) and to harbor specific polymorphisms related to cytotoxic lymphocyte antigen 4 (CTLA-4), interleukin 10, and CD4+ CD25+ regulatory T-cells (T-reg; Thio and colleagues, submitted for publication). These genetic studies have been hampered by insufficient power owing to limited numbers of persons with resolved HCV infections from various race and/or ethnicity groups. Access to seropositive blood donors who have tested RNA-negative offers a solution to this problem, and we are now contributing specimens from donors with presumptive resolved infection to these studies.

TABLE 3. Proportion of donations from first-time seropositive donors in a demographic group testing HCV RNA (MP-NAT)-negative or -positive

Demographic group	HCV RNA status* Negative	Positive	p Value
Age (years)			
<25	28 (14.89)	160 (85.11)	
25-34	64 (23.70)	206 (76.30)	
35-44	180 (20.93)	680 (79.07)	
45-54	110 (17.89)	505 (82.11)	
55-64	15 (15.63)	81 (84.38)	
≥65	5 (20.83)	19 (79.17)	0.12
Sex			
Male	232 (18.40)	1029 (81.60)	
Female	170 (21.41)	624 (78.59)	0.10
Race and/or ethnicity			
Asian	4 (8.16)	45 (91.84)	
Black non-Hispanic	38 (14.39)	226 (85.61)	
Hispanic	62 (22.06)	219 (77.94)	
Other non-Hispanic	16 (21.05)	60 (78.95)	
White non-Hispanic	239 (20.73)	914 (79.27)	0.02

* Data are reported as number (%).

TABLE 4. OR comparing odds of being HCV-seropositive and RNA (MP-NAT)-negative by demographic characteristic

Demographic characteristic	Unadjusted OR (95% CI)	Adjusted OR* (95% CI)
Age (years)		
<25	1.0	1.0
25-34	1.78 (1.09-2.90)	2.08 (1.20-3.60)
35-44	1.51 (0.98-2.33)	1.75 (1.07-2.87)
45-54	1.24 (0.79-1.95)	1.48 (0.88-2.46)
55-64	1.06 (0.54-2.09)	1.30 (0.62-2.73)
≥65	1.50 (0.52-4.36)	1.98 (0.66-5.96)
Sex		
Male	1.0	1.0
Female	1.21 (0.97-1.51)	1.14 (0.90-1.45)
Race and/or ethnicity		
White non-Hispanic	1.0	1.0
Asian	0.34 (0.12-0.96)	0.36 (0.13-1.01)
Black non-Hispanic	0.64 (0.44-0.93)	0.66 (0.45-0.96)
Hispanic	1.08 (0.79-1.49)	1.08 (0.78-1.48)
Other	1.02 (0.58-1.80)	1.03 (0.58-1.83)

* Adjusted for age, sex, and race and/or ethnicity.

Several groups have reported that HCV clearance rates correlate with sex (more frequent clearance in women than men) and age at time of infection (young donors resolve infection more frequently than older donors). In our analysis, although nonsignificant, the odds for women to clear viremia appeared 21 percent greater than for men ($p > 0.05$), similar to the findings in several other studies (reviewed in Orland et al.[1]). The association with age was not significant, and no clear trend was observed.

Our results confirm that HCV RNA positivity is associated with elevation of ALT in RIBA-positive donors. Sixteen percent of viremic seropositive donors had ALT elevations, including 13 percent with elevations greater than the high donor screening cutoff of 120 IU per L. This indicates that a substantial subset of viremic donors have active liver disease at the time of donation. Because liver disease in HCV infection is intermittent and follow-up studies of HCV-infected persons with normal ALT have demonstrated low rates of progression to clinical disease, viremic donors without ALT elevation at the time of donation also warrant clinical follow-up to evaluate prognosis and available therapeutic options.[27] In other analyses, we have shown that HCV RNA-negative, RIBA-positive donors (first-time and repeat donors) have a distribution of ALT levels that is essentially identical to that of HCV ELISA–nonreactive donors.[6] This supports the conclusion that the large majority of RNA-negative, anti-HCV–positive donors have no residual hepatic inflammation and have likely eradicated HCV infection. In this data set, the frequency of elevated ALT among RIBA-positive, first-time donors who tested RNA-negative was 1.5 percent, which is slightly higher than rates of ALT elevation among all HCV ELISA–nonreactive donors. This likely relates to the possibility that a minority of these donors still have active HCV infection that is undetectable by a single RNA determination performed in a MP format of 16 to 24 donations.

Our findings of a lower frequency of HCV RNA positivity in seroconverting repeat donors than in seropositive first-time donors was unexpected. We had hypothesized that seroconverters, on average, would have been more recently infected than first-time donors, and if sampled within the first 6 months after the identification of their RNA positivity, would have retained higher rates of viremia not having adequate time for clearance. We observed that 56 percent of seroconverters tested RNA-negative on their first antibody-positive donation, in contrast to 20 percent in first-time donors, with no apparent relationship between viral clearance and interdonation intervals. It is unknown whether these donors have resolved infection or represent individuals with transient low-level viremia that may only be detected intermittently. Of the 34 seroconverting donors identified in our study, 24 (71%) had interdonation intervals exceeding 12 months so that these donors would not have been expected to have different HCV RNA-positive frequencies than first-time donors. The lower percentage of viremia in repeat seropositive donors could relate to recent observations in chimps and humans that in early infection, as the immune system tries to control the infection, RNA levels fluctuate and may be intermittently nondetectable by MP NAT despite eventual development of chronic infection.[9,11] With time, either the immune system is able to eradicate the infection or the virus escapes. Also, demographic differences in first-time and repeat donors may explain the different RNA-positive frequencies observed in these two groups.

This study has several limitations. We used routine donor screening MP-NAT results, resolved to the individual donation level for NAT-reactive pools, to classify RIBA-

positive donors as RNA-positive or -negative. Owing to the 16- to 24-fold dilution factor inherent in MP-NAT, we may have failed to detect viremia in a proportion of seropositive donors with chronic infection (whether these donors were first-time or repeat donors or with interdonation intervals exceeding 12 months). The MP-NAT assays employed in donor screening, however, have 50 percent detection limits of approximately 200 gEq to mL, equivalent to that of commercially available quantitative HCV RNA assays that are widely used clinically to define viremia status of seropositive persons. We have retested specimens from MP-NAT-nonreactive, RIBA-positive donors individually and have identified HCV RNA positivity in 2 to 5 percent of cases; results from follow-up studies of such donors indicate that they have normal ALT levels and intermittent low-level viremia detectable when samples are tested without dilution.[28]

Finally, our current analysis was limited to demographic and laboratory data available from routine donor screening on approximately 8 percent of the US blood supply during the study period; the REDS centers do not include centers from the southeastern United States where HCV incidence and prevalence are higher than in the rest of the country (data from the ARC national database). We also did not recall donors to investigate risk factors or probable dates of infection, nor did we perform additional laboratory studies to confirm viremia status or further characterize the donors (e.g., HLA typing, cellular immune responses) and the virus (subtype, quasispecies diversity). We have recently begun a study that involves enrollment and follow-up of seropositive donors with resolved infections and matched control donors with persistent infections, as well as NAT-positive antibody-negative donors who are being followed prospectively through seroconversion to establish their resolution status. This study will hopefully contribute to the understanding of determinants of HCV clearance, as well as yield information to assist in counseling and clinical management of the several thousand HCV-infected donors identified annually by US blood centers.[29]

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REFERENCES

1. Orland JR, Wright TL, Cooper S. Acute hepatitis C [review]. Hepatology 2002;33:321-7.
2. Marcellin P. Hepatitis C. the clinical spectrum of the disease [review]. J Hepatol 1999;31(Suppl 1):9-16.
3. Busch MP. Insights into the epidemiology, natural history and pathogenesis of hepatitis C virus infection from studies of infected donors and blood product recipients. Transfus Clin Biol 2001;8:200-6.
4. Dodd RY, Notari EP 4th, Stramer SL. Current prevalence and incidence of infectious disease markers and estimated window-period risk in the American Red Cross blood donor population. Transfusion 2002;42:975-9.
5. Wang B, Schreiber GB, Glynn SA, et al. Does prevalence of transfusion-transmissible viral infections reflect corresponding incidence in United States blood donors? Transfusion 2005;45:1089-96.
6. Stramer SL, Glynn SA, Kleinman SH, et al. Detection of HIV-1 and HCV infections among antibody-negative blood donors by nucleic acid amplification testing. N Engl J Med 2004;351:760-8.
7. Murphy EL, Bryzman SM, Glynn SA, et al. Risk factors for hepatitis C virus infection in United States blood donors. NHLBI Retrovirus Epidemiology Donor Study (REDS). Hepatology 2000;31:756-62.
8. Orton SL, Stramer SL, Dodd RY, Alter MJ. Risk factors for HCV infection among blood donors confirmed to be HCV RNA-positive, anti-HCV non-reactive. Transfusion 2004; 44:275-81.
9. Prince AM, Pawlotsky JM, Soulier A, et al. HCV replication kinetics in chimpanzees with self-limited and chronic infections. J Viral Hepat 2004;11:236-42.
10. Jauncey M, Micallef JM, Gilmour S, et al. Clearance of hepatitis C virus after newly acquired infection in injection drug users. J Infect Dis 2004;190: 1270-4.
11. Cox AL, Netski DM, Mosbruger T, et al. Prospective evaluation of community-acquired acute hepatitis C. Clin Infect Dis 2005;40:951-8.
12. Zavaglia C, Belli LS, Alberti AB, Ideo G, Silini E. Both immunogenetic factors and HCV genotype can influence the outcome of chronic HCV infection. Am J Gastroenterol 1997;92:725-6.
13. Farci P, Shimoda A, Coiana A, et al. The outcome of acute hepatitis C predicted by the evolution of the viral quasispecies. Science 2000;288:339-44.

14. Sheridan I, Pybus OG, Holmes EC, Klenerman P. High-resolution phylogenetic analysis of hepatitis C virus adaptation and its relationship to disease progression. J Virol 2004;78:3447-54.
15. Laskus T, Wilkinson J, Gallegos-Orozco JF, et al. Analysis of hepatitis C virus quasispecies transmission and evolution in patients infected through blood transfusion. Gastroenterology 2004;127:764-76.
16. Mas A, Ulloa E, Bruguera M, et al. Hepatitis C virus population analysis of a single-source nosocomial outbreak reveals an inverse correlation between viral load and quasispecies complexity. J General Virol 2004;85:3619-26.
17. Ray SC, Wang YM, Laeyendecker O, et al. Acute hepatitis C virus structural gene sequences as predictors of persistent viremia: hypervariable region 1 as a decoy. J Virol 1999; 73:2938-46.
18. Thomas DL, Astemborski J, Rai RM, et al. The natural history of hepatitis C virus infection: host, viral, and environmental factors. JAMA 2000;284:450-6.
19. Thio CL, Thomas DL, Goedert JJ, et al. Racial differences in HLA class II associations with hepatitis C virus outcomes. J Infect Dis 2001;184:16-21.
20. Thio CL, Gao X, Goedert JJ, et al. HLA-Cw*04 and hepatitis C virus persistence. J Virol 2002;76:4792-7.
21. Sugimoto K, Stadanlick J, Ikeda F, et al. Influence of ethnicity in the outcome of hepatitis C virus infection and cellular immune response. Hepatology 2003;37:590-9.
22. Grant PR, Busch MP. Nucleic acid amplification technology methods used in blood donor screening [review]. Transfus Med 2002;12:229-42.
23. Giachetti C, Linnen JM, Kolk DP, et al. Highly sensitive multiplex assay for detection of human immunodeficiency virus type 1 and hepatitis C virus RNA. J Clin Microbiol 2002;40:2408-19.
24. Rehermann B, Nascimbeni MO. Immunology of hepatits B virus and hepatitis C virus infection. Nature Rev Immunol 2005;5:215-29.
25. Cooper S, Erickson AL, Adams EJ, et al. Analysis of a successful immune response against hepatitis C virus. Immunity 1999;10:439-49.
26. Diepolder HM, Gerlach JT, Zachoval R. Immunodominant CD4+ T-cell epitope within nonstructural protein 3 in acute hepatitis C virus infection. J Virol 1997;71:6011-9.
27. Strader DB, Wright T, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C [published erratum appears in Hepatology 2004;40:269]. Hepatology 2004;39:1147-71.
28. Tobler LH, Murphy EL, Andrews WW, et al. Follow-up study of HCV seropositive blood donors who tested positive or negative for HCV RNA by minipool-NAT screening. Transfusion 2005;45:92A (abstract SP208).
29. Busch MP, Page-Shafer KA. Acute-Phase hapititis C virus infection: Implications for research, diagnosis, and treatment. Clin Infect Dis 2005;40:959-61.