HIV antibody screening remains indispensable for ensuring viral safety of blood components despite NAT implementation

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BACKGROUND: The main objective of the implementation of NAT for the screening of blood-borne viruses was to compensate for the failure of serologic assays during the window period. Because this new screening procedure theoretically covers the entire period of infectivity, the necessity for maintaining serologic assays in blood screening strategy could become questionable.
STUDY DESIGN AND METHODS: To investigate this issue, a panel of 35 samples has been studied by NAT. These samples had been collected from HIV-1 antibody-positive individuals presenting a persistently low viral RNA load (<400 copies/mL) in the absence of antiviral therapy. All samples were analyzed with the minipool (×8) NAT routinely used in blood bank setting (HIV-1 and HCV assay based on transcription-mediated amplification) and with single-donation testing.
RESULTS: The minipool NAT failed to detect the presence of HIV RNA in 15 of the 35 samples (11 remained negative when retested). Single-donation testing gave negative results in 4 samples (3 remained negative when retested). Fourteen of the 18 samples with a viral load greater than 50 copies per mL were positive by minipool NAT versus 6 of the 17 samples with fewer than 50 copies per mL ($p = 0.02$).
CONCLUSION: The results clearly demonstrate that anti-HIV screening should not be withdrawn from biologic qualification procedures of blood donations, even when single NAT is performed.

Before the implementation of NAT in blood donation screening in France, the residual risk of transfusion-transmitted viral infections during the window period[1] was estimated at 1 in 870,000 and 1 in 1,400,000 donations for HCV and HIV, respectively.[2] To further reduce this risk, the French Health Authorities have implemented NAT for the screening for these two viruses by testing donations in minipools. Several countries have adopted the same strategy for HCV only[3] or for both HCV and HIV.[4-6]

Since its implementation, HCV and HIV NAT has proven efficient in improving transfusion safety compared to screening based on the antibody assay only.[5,7-9] For HIV, viral RNA detection theoretically ensures virus detection during both the window period and the antibody-positive phase.[10] Antibody-based assays are capable of detecting virus during the chronic phase only.[11] For this reason, the value of antibody assay for blood safety could be evaluated and its usefulness in systematic blood donation screening could be challenged.

Recently, a French blood donor tested positive for the presence of HIV antibody and negative for the presence of viral RNA by minipool (×8) NAT. This sample contained a very low viral load, subsequently found at 27 copies per mL of HIV RNA through a testing on an undiluted sample (unpublished data, communicated by P. Morel). In the absence of systematic HIV antibody screening, such a

ABBREVIATION: TMA = transcription-mediated amplification.

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blood donation could have led to the infection in one or more recipients.

To demonstrate that HIV antibody screening remains an important procedure in blood safety, even after the implementation of NAT, we have undertaken a study for evaluating the limits of NAT in detecting HIV infections. We investigated serum samples collected from untreated HIV-1-infected individuals presenting a very low viral load and who were previously diagnosed as anti-HIV+ through systematic screening of blood donations.

MATERIALS AND METHODS

Samples were obtained from a cohort of individuals who tested positive for the presence of HIV antibody through the systematic screening of blood donations, who were being followed in our outpatient clinic on regular visits. This follow-up consisted of a physical examination for determination of the patient's clinical status (according to the CDC classification[12]) and blood specimen collection for laboratory evaluation. Serum samples were frozen at –80°C within 4 hours of collection. HIV infection status had been ascertained by two positive results with an ELISA and a confirmed HIV-1+ Western blot assay. In this cohort of HIV+ donors, we focused on subjects subsequently recognized as long-term nonprogressors (defined as individuals belonging to CDC A stage and maintaining a stable CD4+ T-cell count despite at least a decade of seropositivity[13,14]). Subjects presenting, on several consecutive samples, a viral load lower than 400 copies per mL (as determined in a previous study[13]) in the absence of any therapy for HIV infection were selected for the study. HIV viral loads were tested once more with a licensed HIV test (Amplicor HIV-1 Monitor test, Version 1.5 [ultrasensitive], Roche Diagnostics Systems, Branchburg, NJ) with an unfrozen aliquot. This procedure has an analytical sensitivity for detection of HIV-1 RNA in plasma at 20 copies per mL.[15] Six individuals (four men and two women) were selected. The mean age at the discovery of HIV infection was 27 years (range, 21-32 years). Risk factors of HIV infection were homosexual contacts (n = 4) and intravenous drug addiction (n = 2). The HIV serotype[16] was B in the six individuals. All individuals were at the CDC A of CDC classification at the end of the follow-up period. The panel included 35 samples (kept frozen until the study) collected from these six individuals. The biologic characteristics of each sample are given in Table 1. Three HIV antibody-negative samples collected from individuals at low risk of HIV infection and five HIV-antibody-positive samples with a viral load higher than 400 copies per mL were used as negative and positive controls, respectively.

The 35 HIV antibody-positive samples and the eight control samples were coded for a blind study and subjected to a routine run to NAT according to one of the two procedures routinely used in French blood banks (HIV-1 and HCV assay based on transcription-mediated amplification [TMA]). Each sample was assayed in both an eight-pool-size and a single-donation testing at the same time. The minipool testing negative samples and the single-donation testing negative samples were tested on a new aliquot of the same sample.

Sample pool was assembled by adding 2100 µL of negative plasma collected from a single donor to 300 µL of the infected sample. The pooling was conducted by an automated distributor (Genesis RSP1560, Biomerieux, Marcy-L'Etoile, France). After mixing, 500 µL was used for analysis. The panel was subjected to the HIV-1 and HCV TMA (developed by Gen-Probe, Inc., San Diego, CA; and distributed as the Procleix HIV-1 and HCV assay by Chiron Corp., Emeryville, CA). This assay is currently utilized for large-scale screening of pooled samples.[17] Extraction procedure of both HCV and HIV-1 RNA is based on magnetic microparticle-assisted oligocapture. The analytical sensitivity of the TMA assay was estimated at 100 copies per mL HIV-1 RNA in the single-donation testing format.[18-20] During the validation study performed in France before NAT implementation, the 100 percent detection limit of HIV RNA in eight-size minipools was evaluated at 400 copies per mL (A. Assal and J. Coste, personal communication).

Proportions of positive results according to the viral load in the studied samples were compared with the chi-square test with Yates correction when necessary. Differences were considered significant when $p < 0.05$.

RESULTS

Testing of the sample panel

Results for the negative and positive controls were as expected with single-donation testing and minipool NAT. Among the 35 HIV antibody-positive samples, 15 (42.8%) (belonging to four patients) were minipool NAT-negative, and 4 (11.4%) (belonging to three patients) were single-donation testing-negative. These 4 samples were minipool NAT-negative (see details in Table 1).

Four of the 15 minipool NAT-negative samples became positive when tested in a subsequent assay (these 4 samples had given a single NAT-positive result). One of 4 single donation testing-negative samples was single donation testing positive when retested on a new aliquot (see Table 1).

Results of the panel testing according to the viral load

Minipool NAT. Fourteen the 18 (78%) samples containing a viral load higher than 50 copies per mL were minipool NAT-positive versus 6 of the 17 (35%) samples with a viral load below 50 copies per mL ($p = 0.02$).

Single-donation testing. Fourteen of the 17 samples (82%) with a viral load lower than 50 copies per mL as well

TABLE 1. Results of minipool (eight-pool size) and single-donation testing HIV RNA NAT blood screening in 35 serum samples with an HIV RNA load of lower than 400 copies per mL

Patient	Date	Delay from the first visit (months)	CD4+ T-cell count (/mL)	HIV RNA load* (copies/mL)	Minipool NAT	Minipool NAT (second round)	Single-donation testing	Single-donation testing (second round)
1	December 1986	0	780	130	Negative	Positive	Positive	
	April 1988	16	740	60	Negative	Negative	Negative	Negative
	February 1989	26	630	<20	Negative	Negative	Positive	
	May 1990	42	660	300	Negative	Negative	Positive	
	January 1992	62	640	<20	Negative	Negative	Positive	
	July 1993	79	641	66	Positive		Positive	
	June 1994	90	716	143	Positive		Positive	
	April 1995	101	655	147	Positive		Positive	
2	October 1986	0	470	21	Positive		Positive	
	April 1987	61	420	28	Positive		Positive	
	February 1988	16	390	<20	Negative	Negative	Positive	
	August 1988	22	470	<20	Negative	Negative	Negative	Negative
	February 1989	28	560	<20	Negative	Negative	Negative	Positive
	September 1989	35	700	<20	Positive		Positive	
	March 1990	40	520	20	Negative	Negative	Positive	
	February 1991	52	560	26	Negative	Positive	Positive	
	March 1992	64	660	117	Positive		Positive	
3	July 1986	0	627	24	Positive		Positive	
	April 1987	9	916	<20	Positive		Positive	
	May 1988	21	640	33	Positive		Positive	
	April 1991	56	520	247	Positive		Positive	
4	May 1986	0	220	239	Positive		Positive	
	December 1987	19	240	46	Negative	Positive	Positive	
	May 1988	24	340	383	Positive		Positive	
	December 1989	43	320	71	Positive		Positive	
	July 1990	50	260	81	Negative	Positive	Positive	
5	May 1986	0	379	135	Positive		Positive	
	February 1987	8	332	97	Positive		Positive	
	June 1988	25	240	330	Positive		Positive	
	October 1989	41	160	79	Positive		Positive	
6	September 1986	0	556	23	Negative	Negative	Positive	
	October 1987	13	470	<20	Negative	Negative	Negative	Negative
	May 1990	44	590	33	Negative	Negative	Positive	
	July 1993	82	615	351	Positive		Positive	
	October 1995	109	694	120	Positive		Positive	

* Amplicor HIV-1 Monitor test, version 1.5 (ultrasensitive procedure).

as 17 of the 18 (94%) samples with a viral load higher than 50 copies per mL were positive by single-donation testing (nonsignificant difference).

DISCUSSION

Whereas HIV NAT was implemented in the blood bank setting with the main objective of compensating for the failure of serologic assays during the window period, the introduction of this procedure, thought to cover the entire period of HIV infectivity including the window period, could challenge the necessity of maintaining serologic assays in blood screening. To investigate this issue, we have analyzed a panel of samples previously tested positive for anti-HIV-1 and presenting a low viral load in the absence of any antiviral therapy, with the routine HIV RNA NAT. The results indicate that minipool NAT could fail to detect a substantial proportion of samples (43% in our series). Single HIV RNA NAT assays could also fail detection, however, in a much lower proportion (11%). Obviously, the benefit of single-donation testing is linked to higher viral load in nondiluted samples. The dilution factor of a minipool procedure can affect the detection of a viral load close to, or lower than, the sensitivity limit of the procedure. The single-donation testing results obtained in our study with the Procleix HIV-1 and HCV assay would have been probably similar with the other NAT procedure implemented in several blood banks of our country (Cobas HIV AmpliScreen 1.5 assay, Roche, in combination with Nuclisens Extractor, BioMérieux). Indeed, a recent international study has shown that the detection limits of both methods are equivalent.[19]

The benefit of single-donation testing versus minipool testing could be predicted from the results of a validation study conducted in France just before the implementation of NAT in blood banks (A. Assal and J. Coste, personal communication). In this study, the 100-percent detection limits on an HIV-1 RNA reference sam-

ple were at 50 copies per mL for the TMA HIV-1 and HCV assay on an undiluted plasma sample; the 100 percent detection limits were at 400 copies per mL for the TMA HIV-1 and HCV assay in an eight-pool size. Our present data are thus in agreement with the results of the validation study.

The six individuals from whom samples were collected for this study had been qualified several years before as blood donors by clinical selection before donation; obviously, their blood donation had been discarded as a result of HIV antibody screening. But if blood screening had been based on minipool NAT only, four of the six individuals might not have been detected as HIV-infected, and each of them could have given blood several times, which in turn could have led to HIV infection of several recipients. Our study strongly argues that the withdrawal of HIV antibody screening could seriously compromise the safety of blood components, even when single-donation NAT is to replace minipool procedure. Another crucial argument in favor of maintaining of HIV antibody screening is related to the failure of the current NAT assays to detect HIV-2 RNA.

Since the implementation of HIV RNA NAT in the French blood banks (July 1, 2001), 2 antibody-negative, NAT-positive blood donations; 42 antibody-positive, NAT-positive blood donations; 1 antibody-positive, NAT-negative blood donation; and 1 antibody-negative, NAT-negative blood donation infectious in a recipient have been observed throughout a 15-month period and among more than 3 million blood donations (French Blood Center, unpublished data). Such results confirm that despite the exceptional occurrence of antibody-positive, NAT-negative blood donations, the licensed serologic tests and NAT are not equivalent but complementary for ensuring the safety of HIV blood screening. In our view, it is not conceivable today to withdraw the antibody assay on the pretext that NAT has been implemented.

Nevertheless, viral screening strategy of blood donations could be further debated when procedures of pathogen inactivation of blood components are considered as efficient measures to ensure blood safety.[21-23] Indeed, one would prefer an efficient inactivation of all pathogens (virus or bacteria, identified or not) to an efficient genomic detection of known infectious agents.

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