A pilot study on screening blood donors with individual-donation nucleic acid testing in China

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Background. Nucleic acid amplification testing (NAT) is not yet obligatory in China for blood donor screening and the risk of enzyme immunoassay (EIA)-negative, NAT-reactive donations in Chinese blood donors has rarely been reported. The aim of this study was to screen a population of Chinese blood donors using a triplex individual-donation (ID)-NAT assay and assess the safety benefits of implementing NAT.

Materials and methods. Between 1st August, 2010 and 31st December, 2011 all donations at a Chinese blood centre were screened individually using the Procleix® Ultrio® assay, a multiplex NAT assay for the detection of hepatitis B virus (HBV) DNA, hepatitis C virus (HCV) RNA and human immunodeficiency virus-1 (HIV-1) RNA. All donations were also screened for HBsAg, anti-HIV and anti-HCV using two different EIA for each marker. Samples with discordant results between NAT and EIA were further tested with an alternative NAT assay (Cobas® TaqMan®). Potential yield cases (serologically negative/NAT-reactive donors) were further evaluated when possible.

Results. During the study period a total of 178,447 donations were screened by NAT and EIA, among which 169 HBV NAT yield cases (0.095%) were detected. No NAT yield cases were found for HIV-1 or HCV. For the HBV NAT yield cases, follow-up results showed that 11 (6.51%) were probable or confirmed HBV window period infections, 5 (2.96%) were chronic HBV carriers and 153 (90.53%) were probable or confirmed occult HBV infections. There was a statistically significant difference between the NAT-positive rates for first-time *vs* repeat donations (0.472% *vs* 0.146%, respectively; P<0.001).

Discussion. Our data demonstrate that the potential HBV yield rate was 1:1,056 for blood donations in the Zhejiang province of China. Implementation of NAT will provide a significant increment in safety relative to serological screening alone.

Keywords: nucleic acid amplification test, enzyme immunoassay, blood screening.

Introduction

Routine blood donation screening by nucleic acid amplification testing (NAT) has been introduced in many countries in Europe, North and South America, Southeast Asia as well as South Africa and Australia[1-7]. Many reports have now been published on the sensitivity and specificity of NAT assays as well as the prevalence of serologically negative/NAT-reactive (NAT yield) cases. As these reports have come from countries that differ with respect to the epidemiology of blood-borne pathogens, they show dramatic differences in the prevalence of NAT yield and occult infections. Blood donation screening by NAT is not compulsory in mainland of China although it is routinely performed in a few Chinese cities. It has been reported that occult hepatitis B virus (HBV) infection (OBI) is rare in regions with a low prevalence of HBV in the general population, but there is little information about OBI rates in regions with high a prevalence of HBV such as the Asia/Pacific region[8]. A high prevalence of HBV infection has also been reported in the Chinese population. In the 1980s the rate of HBsAg-positivity was 10.0% and, despite the widespread use of hepatitis B vaccine and other strategies to control HBV infection, the rate of HBsAg carriers was still reported to be 7.2% in 2006[8-11]. However, given the limited screening of blood donors by NAT, further analysis is required to establish a reliable estimate of the prevalence of NAT yield donors in mainland China. In this study, we evaluated the impact of screening blood donations by NAT in the blood centre of Zhejiang province which is located in the southeast of mainland of China. This study provides further information about the prevalence of NAT yield cases in the donor population of mainland China.

Materials and methods

Specimen collection

All samples were collected from voluntary unpaid donors with informed consent at the blood centre of Zhejiang province, China, between 1st August, 2010 and 31st December, 2011. Three blood specimens were collected from each donor, one for HBV surface antigen (HBsAg), anti-hepatitis C virus (HCV), anti-human immunodeficiency virus (HIV), and *Treponema pallidum* antibody (anti-TP) screening by enzyme immunoassay (EIA), one for ABO grouping and alanine aminotransferase (ALT) testing and one for individual donation (ID)-NAT screening. Specimens were collected, stored and handled according to the assay manufacturers' instructions.

Pre- and post-donation screening of routine blood donors

According to the guidelines for blood donations in China, all donors are asked to fill in a risk factor questionnaire to exclude those at risk of exposure to transfusion-transmissible infections. Those considered as safe donors were physically examined by a doctor prior to acceptance for donation. In the blood centre of Zhejiang province, all donors were pre-screened (i.e. prior to donating) for HBsAg with a rapid HBsAg assay (colloidal gold strip method, Xiamen Xinchuang Company, Xiamen, China) and deferred if positive. ALT testing (Reflotron system, Roche diagnostics Company, Shanghai, China) was also performed prior to donating and donors were deferred from donating if their ALT was abnormal (acceptance criteria: ≤40 IU/L; deferral criteria: >40 IU/L).

Following pre-donation screening, qualified donors provided a total of 200, 300 or 400 mL whole blood (200 mL=1 unit) or an apheresis platelet donation. Following donation, donor blood samples were tested for HBsAg, anti-HCV, anti-HIV and anti-TP by EIA, the ALT level was assayed and ABO blood grouping was performed twice, using reagents from two different manufacturers. According to state regulations in China, all donations should be screened for HBsAg, anti-HCV and anti-HIV with two EIA from different manufacturers. One round of screening was performed using EIA from Chinese manufacturers for HBsAg (Xiamen Xinchuang Company, Xiamen, China), anti-HCV (Xiamen Xinchuang Company, Xiamen, China), anti-HIV (Beijing Wantai Company, Beijing, China). The second round of screening was performed using imported EIA for HBsAg (Abbott Murex, Dartford, UK), anti-HCV (Ortho-Clinical Diagnostics, Raritan, NJ, USA), anti-HIV (bioMérieux, Marcy l'Etoile, France). Samples that were reactive on either of the two EIA for each viral marker were defined as positive for that marker.

All assays were performed according to the manufacturers' instructions.

Nucleic acid amplification technology

Samples from individual donations were tested in parallel with qualitative detection of HIV-1, HCV, and HBV by the multiplex Procleix® Ultrio® Assay (Novartis Diagnostics, Emeryville, CA, USA) and the Procleix discriminatory assays for HBV DNA, HCV RNA, and HIV-1 RNA (dHBV, dHCV, and dHIV). The Procleix assays were performed on a Procleix Tigris instrument (Novartis Diagnostics, Emeryville, CA, USA) according to manufacturer's instruction. The analytical sensitivities of the Procleix® Ultrio® assay for HBV DNA, HCV RNA and HIV-1 RNA are 10.4 (9.2-12.2) IU/mL, 3.0 (2.7-3.4) IU/mL, and 47.9 (43.3-54.5) IU/mL, respectively. The analytical sensitivities for the Procleix dHBV, dHCV, and dHIV assays are 8.5 (7.6-9.8) IU/mL, 3.2 (2.8-3.6) IU/mL and 53.6 (47.9-61.2) IU/mL, respectively. Samples that initially tested reactive on the Ultrio® assay were retested in duplicate on the same assay. If one or both duplicate retests were reactive, the sample was tested on the Procleix discriminatory assays for HBV DNA, HCV RNA, and HIV-1 RNA to identify the specific agent. The samples which were initially reactive and repeatedly reactive in the Ultrio® assay were defined as positive for the Procleix® Ultrio® assay. The samples which were initially reactive but then repeatedly non-reactive in the Ultrio® assay were defined as negative for the Procleix® Ultrio® assay.

Follow-up study of hepatitis B virus NAT-reactive, EIA-negative donors

All EIA-negative, NAT-reactive samples from donors were investigated with alternative NAT and supplemental serological tests. No NAT yield cases were found for HIV-1 or HCV. HBV NAT yield cases were tested for viral load using the Roche Cobas AmpliPrep with real-time polymerase chain reaction on a Cobas TaqMan analyser (Roche Diagnostics Company, Shanghai, China). The manufacturer states that the lower limit of detection for the HBV DNA assay is 20 IU/mL. This HBV test targets a different region of the genome and can, therefore, be considered as an alternative NAT for confirmation of Ultrio/dHBV reactive results. Supplemental serological tests for the NAT-yield samples were HBsAg, antibodies to HBsAg (anti-HBs), hepatitis B E antigen (HBeAg), antibodies to HBeAg (anti-HBe) and antibodies to hepatitis B core antigen (anti-HBc), which were all tested by electroluminescence assays with a Cobas e601 analyser (Roche Diagnostics Company, Shanghai, China).

Confirmatory testing

HBsAg and anti-HCV reactive tests among blood donations were confirmed by, respectively, a Murex HBsAg neutralisation test (Abbott Murex, Dartford, UK) and a recombinant immunoblot assay (RIBA, Ortho-Clinical Diagnostics, Raritan, NJ, USA) according to the manufacturers' instructions. Anti-HIV reactive samples were confirmed by a western-blotting method by the Centre of Disease Control of Zhejiang province, in accordance with state regulations of China.

Statistical analysis

Statistical analyses were performed with SPSS 16.0 software (SPSS Inc., Cary, NC, USA). Differences in the rate of NAT-positive donations, NAT-yield donations and EIA-positive donations between first-time donor and repeat donors were analysed with the chi-square test. P values less than or equal to 0.05 were considered statistically significant.

Results

Comparison of serological and NAT results

All potential blood donors between 1st August, 2010 and 31st December, 2011 were pre-screened for HBsAg status by a rapid HBsAg assay: 2,197 (1.216%) were found to be positive and were, therefore, deferred from donation. During this same period a total of 178,447 donations were made by donors who were HBsAg-negative in the pre-screening test and underwent further investigations. Among the178,447 donations, 1267 (0.710%) gave discrepant EIA and NAT results. It was found that 169 (0.095%) were EIA-non-reactive/NAT-reactive and 1,098 (0.615%) were EIA-reactive/NAT-non-reactive. A further 417 (0.234%) donations were both EIA- and NAT-reactive while the remaining 176,763 (99.056%) were both EIA- and NAT-non-reactive.

A total of 1,515 donations were EIA-reactive (1,098 NAT-non-reactive and 417 NAT-reactive). Of the 829 donations that were HBsAg EIA-reactive, neutralisation testing was performed on 746, with 88.9% (663/746) being confirmed as positive. HBsAg confirmatory testing could not be performed on the remaining 83 donations, which were reactive only on a Chinese EIA, due to the lack of an appropriate confirmatory assay. Five hundred and seventeen donations were anti-HCV EIA-reactive of which 103 (19.9%) were confirmed positive by RIBA and 130 (25.2%) were indeterminate. With regards to the anti-HIV screening, 169 donations were EIA-reactive of which 28 (16.6%) were confirmed positive by western blotting. Samples that were reactive on EIA from different manufacturers for HBsAg or anti-HIV were highly correlated with positive results by confirmatory testing (Table I). Some donations that were reactive on one anti-HCV EIA but not reactive on the second EIA were confirmed positive by RIBA which indicates some differences in sensitivity between anti-HCV screening assays. In addition, some of the anti-HCV confirmed positive donations were non-reactive in the NAT assay.

NAT-positive samples among 178,447 blood donations

The most frequent infectious disease marker among the 178,447 blood was HBV-DNA, which was present in 469 (0.263%) donations of which 133 were HBsAg negative (Table II). HCV-RNA was detected in 41 (0.023%) donations and HIV-RNA in 28 (0.016%) donations. All HCV-RNA and HIV-RNA positive donations were also antibody reactive. A further 48 (0.027%) donations were Ultrio® repeat reactive but non-reactive on all three Procleix discriminatory assays; 12 of these donations were HBsAg reactive and all of them were positive for anti-HBc. The overall prevalence of NAT positivity in blood donors was 0.329% (586/178,447).

Table I - Distribution of EIA-positive samples.

Test	EIA-positive		Confirmatory test			NAT	
			+	±	−	+	−
HBV	D*+/ I&−	83		ND		0	83
	D−/ I+	169	89	60	20	41	128
	D+/ I+	577	574	3	0	307	270
HCV	D+/ I−	139	11	29	99	1	138
	D−/ I+	254	24	72	158	3	251
	D+/ I+	124	68	29	27	37	87
HIV	D+/ I−	14	0	0	14	0	14
	D−/ I+	127	0	2	125	0	127
	D+/ I+	28	28	0	0	28	0
Total		1,515	794	195	443	417	1,098

*D: domestic reagent; &I: imported reagent; D+ or I+: D or I positive; ND: not done; ±: indeterminate.

Table II - The distribution of NAT-positive donations.

Test	NAT-positive	NAT-positive rate	EIA	
			+	−
HBV-DNA	469	0.263%	336	133
HCV-RNA	41	0.023%	41	0
HIV-RNA	28	0.016%	28	0
Non-discriminated*	48	0.027%	12 (HBsAg)	36
Total	586	0.329%	417	169

*: Ultrio repeat reactive but discriminatory Procleix® Ultrio® NAT tests for HBV DNA, HCV RNA, and HIV-1 RNA were non-reactive. +: positive, −: negative.

Supplemental HBV serological testing and alternative NAT assay of the potential HBV NAT-yield cases

No NAT yield cases were found for HIV-1 or HCV. In contrast 169 (0.095%) HBV NAT yield cases were detected in the 178,447 blood donations.

Among the 169 HBV NAT yield cases (Table III), five cases were weakly HBsAg positive by alternative HBsAg testing with Cobas e601, showing low HBV viral loads (Table IV). While these five cases could be classified as low-level chronic HBV carriers, they should be considered as true NAT yield cases since they were not detected by the routine HBsAg screening tests.

Ninety-four of the potential HBV NAT yield cases were classified as confirmed OBI HBV NAT yield cases based on the detection of HBV DNA by an alternative NAT assay (Table III). Fifty-two of the potential HBV-yield cases were non-reactive with the alternative HBV NAT, non-reactive with the supplemental HBsAg test, but reactive with the anti-HBc and/or anti-HBs tests and were classified as probable OBI HBV-yield cases. Seven of the potential HBV-yield cases were negative in the supplemental HBsAg test and reactive for anti-HBc, indicating a past history of HBV infection, but an alternative HBV NAT and other HBV serological markers were not tested because of an insufficient sample volume; these cases were also classified as probable OBI HBV NAT yield cases. In total, 59 of all potential HBV-yield cases were classified as probable OBI HBV-yield cases.

The remaining 11 potential HBV-yield cases were non-reactive by supplemental HBV serological tests (HBsAg, anti-HBs, HBeAg, anti-HBe and anti-HBc assay by electroluminescence assay), but nine of them were reactive in the alternative NAT assay (Table III).

Table III - Results of the supplemental HBV serological test and alternative NAT in potential HBV NAT yield cases.

Classification	N.	ALT NAT#	HBs Ag*	Anti-HBs	HBeAg	Anti-HBe	Anti-HBc
Chronic infection (n=5)	3	+	+	−	−	+	+
	1	+	+	−	−	−	+
	1	+	+	−	+	−	+
Confirmed OBI (n=94)	6	+	−	+	−	−	−
	23	+	−	−	−	−	+
	19	+	−	+	−	−	+
	35	+	−	−	−	+	+
	7	+	−	+	−	+	+
	4	+	−	ND	ND	ND	+
Probable OBI (n=59)	6	−	−	+	−	−	−
	19	−	−	−	−	−	+
	12	−	−	+	−	−	+
	9	−	−	−	−	+	+
	6	−	−	+	−	+	+
	7	ND	−	ND	ND	ND	+
Confirmed WP (n=2)&	1	+	−	−	−	−	−
	1	−	−	−	−	−	−
Probable WP (n=9)	8	+	−	−	−	−	−
	1	−	−	−	−	−	−

#: alternative NAT; *: alternative HBsAg test; &: these samples were confirmed by the presence of HBsAg in the follow-up testing; ND: not done; +: positive, −: negative.

Table IV - Five cases that were EIA-negative and weakly positive for HBsAg by Cobas e601.

Donor	HBsAg (COI)	Anti-HBs	HBeAg	Anti-HBe	Anti-HBc	Alternate NAT (IU/mL)
697	0.975/1.1	−	−	+	+	35.3
610	0.97/1.1	−	−	−	+	<20
102	0.918	−	+	−	+	<20
793	0.973	−	−	+	+	<20
647	0.918	−	−	+	+	<20

+: positive; −: negative.

Two of these 11 potential HBV NAT yield cases were confirmed by the presence of HBsAg, HBV DNA, and/or anti-HBc in the follow-up testing. These two specimens were classified as confirmed window-period HBV NAT yield cases. One of the 11 potential HBV-yield cases was in the recovery phase from HBV infection, and was defined as a probable window-period HBV NAT yield case because this donor was positive for anti-HBc, anti-HBs and anti-HBe, but negative for HBsAg and HBeAg in the follow-up testing. Another eight cases for which samples were not available for follow-up testing were classified as probable window-period HBV NAT yield cases. In total, there were nine probable window-period HBV NAT yield cases.

Therefore, of the 169 potential HBV-yield cases (Table III), 11 (6.51%) were probable or confirmed HBV window period infection, 5 (2.96%) were chronic HBV carriers and 153 (90.53%) were probable or confirmed OBI with 56 cases being anti-HBs reactive. Seven of the 153 OBI cases were not tested with alternative HBV-DNA NAT because of insufficient sample volume. In the remaining 146 OBI cases, 52 (35.62%) were negative in the alternative HBV-DNA NAT, 78 (53.42%) had values of less than 20 IU/mL, 6 (4.11%) had values between 20 IU/mL and 50 IU/mL, and only 10 (6.85%) had values of more than 50 IU/mL in the alternative HBV-DNA NAT.

Donors negative by the alternative NAT assay but positive by the Ultrio® HBV-DNA assay

Of the 169 HBV NAT yield cases, seven cases were not tested by an alternative HBV NAT assay because of insufficient sample volume and 54 (33.3%) of the remaining 162 HBV NAT yield cases were non-reactive on an alternative NAT assay. Eight of these 54 cases were followed up for 2 to 12 months and retested with the Procleix® Ultrio® multiplex assay and an alternative NAT assay, and underwent supplemental HBV serological testing. The mean age of participants was 34.4 years. Among them, 62.5% were women and 50% were first-time donors. Upon follow-up testing, three of the eight cases were reactive on both the Ultrio® multiplex assay and the alternative NAT assay and five cases (four of them were anti-HBs positive) remained non-reactive on the multiplex Ultrio® assay (Table V).

Comparison of the NAT and EIA reactivity rates between first-time and repeat donations

In this study there were 99,484 (55.75%) first-time donations and 78,963 (44.25%) repeat donations (Table VI). Among the 1,515 EIA-reactive donations, 1,259 (83.10%) were first-time donations and 256 (16.90%) were repeat donations. The EIA reactivity rates were 1.27% and 0.32% for first-time and repeat donations respectively; the difference was statistically significant (P<0.001). Of the 586 NAT-positive donations, 470 (80.20%) were first-time donations and 116 (19.80%) were repeat donations. The NAT reactivity rates were 0.472% and 0.146% for first-time and repeat donations, respectively (P<0.001). The rates of HBV-DNA positivity were 0.415% for first-time donations and 0.132% for repeat donations (P<0.001). All of the HCV-RNA-positive cases were first-time donations, with the rate being 0.041%. The HIV-RNA-positive rates were 0.016% and 0.015% for first-time donations and repeat donations, respectively (not significant, P>0.05). Among the 169 HBV NAT yield donations, 83 (49.11%) were first-time and 86 (50.89%) were repeat donations. The HBV NAT yield rates were 0.083% and 0.109% for first-time donations and repeat donations, respectively (not significant, P>0.05).

Discussion

In this study, we evaluated ID-NAT and serological screening of 178,447 donations, which were all donations collected at the Zhejiang province blood centre from 1st August, 2010 to 31st December, 2011. The results of the EIA and NAT assays were discrepant in some donations and the NAT reactivity rate among serologically-confirmed positive donations was 52.52% (417/794), which is lower than the reports from a international survey[12]. This may be due to the analytical sensitivity of the NAT assay we used. For example, the reactivity rate would probably increase if the Procleix Ultrio Plus assay, which has an improved analytical sensitivity (not registered in China), or other more sensitive NAT assay were to be used. However, our data indicate that serological screening should be retained even with the implementation of sensitive ID/minipool-NAT testing.

Table V - The follow-up studies of donors negative by alternative HBV NAT testing but positive by the Ultrio HBV-DNA assay.

Donor	Date	HBsAg	Anti-HBs	Anti-HBc	HBeAg	Anti-HBe	Ultrio#	Alternative& NAT (IU/mL)
042	Nov 14, 2010	−	+	−	−	−	+	−
	May 30, 2011	−	ND	ND	ND	ND	−	ND
792	Jan 14, 2011	−	+	+	−	−	+	−
	Dec 28, 2011	−	ND	ND	ND	ND	−	ND
978	Feb 6, 2011	−	−	+	−	−	+	−
	Aug 16, 2011	−	−	+	−	−	+	<20
849	Feb 12, 2011	−	+	+	−	−	+	−
	Jan 4, 2012	−	ND	ND	ND	ND	−	ND
233	Mar 11, 2011	−	−	+	−	−	+	−
	Sep 10, 2011	−	−	+	−	−	+	<20
951	June 27, 2011	−	+	+	−	−	+	−
	Sep 6, 2011	−	ND	ND	ND	ND	−	ND
900	July 5, 2011	−	−	+	−	−	+	−
	Feb 27, 2012	−	ND	ND	ND	ND	−	ND
901	July 15, 2011	−	−	−	−	−	+	−
	Oct 1,2011	−	−	−	−	−	+	<20

ND: not done; +: positive; −: negative; #: Procleix® Ultrio® assay; &: Cobas TaqMan analyser.

Table VI - The rate of NAT-positive, HBV NAT-yield and EIA-positive donations among first-time donations and repeat donations.

Donor	Number	First-time donations	Repeat donations
Total	178,447	99,484 (55.75%)	78963 (44.25%)
EIA-positive*	1515	1259 (83.10%)	256 (16.90%)
NAT-positive	586	470 (80.20%)	116 (19.80%)
HBV-DNA#	517	413 (79.88%)	104 (20.12%)
HCV-RNA	41	41 (100%)	0
HIV-RNA	28	16 (57.14%)	12(42.86%)
NAT-yield	169	83 (49.11%)	86 (50.89%)

*: EIA includes HBsAg, anti-HCV and anti-HIV; #: non-discriminated cases are included.

The rate of confirmed HBsAg positivity in the donors in our study (at least 0.372%) is substantially higher than that reported in donors in the United States and Europe[5,12]. However, the actual HBsAg-positive rate in the blood donor population of Zhejiang province is higher than that reported in our study as at least 1.216% of HBsAg-positive subjects were excluded from donating by pre-donation testing with a rapid HBsAg assay and some by ALT testing. It has been reported that OBI is rare in regions with low HBV endemicity, but there has been little information from regions with a high prevalence of HBsAg carriers in the general population. We found that the most frequent infectious disease marker detected by NAT screening was HBV-DNA with a prevalence of 0.263%. However, the actual prevalence of HBV-DNA-positive donors may be higher than this reported rate because some donations were Ultrio® repeat reactive but non-reactive on the Procleix discriminatory assays for HBV-DNA, HCV-RNA, and HIV-1-RNA. Among 48 non-discriminated donations, 32 were positive and 16 were negative by the alternative HBV-DNA assay. In addition, 12 of these 48 non-discriminated donations were HBsAg-reactive while all 48 samples were anti-HBc reactive. It is, therefore, expected that the detection rate of HBV-DNA-positive donors would increase with the use of the Procleix Ultrio Plus assay which has an improved sensitivity for HBV-DNA. In China, blood donors are not screened for anti-HBc because the prevalence of this marker is too high for use as a universal screening marker. The detection of OBI in Chinese blood donors is, therefore, dependent on the use of an HBV-NAT assay. We conclude that the rate of HBV-DNA was at least 0.281% (1 per 356 donations, at least 501 confirmed HBV-DNA-positive donations among 178,447 blood donations) in the donor population in this study.

There were 54 samples that were reactive on the Ultrio® assay but non-reactive on the alternative NAT assay. Follow-up of these donors showed the presence of HBV serological marker(s) in 52 of the samples. These discrepant results may reflect differences in the target region of the HBV genome and/or intermittent detection of HBV-DNA due to low viral load[13]. Eight of these 54 donors were followed up 2 to 12 months after their donation. Follow-up samples from five of them were negative by the multiplex Ultrio® NAT assay, probably reflecting a viral load below the limit of detection of the assay.

The rate of potential HBV NAT yield cases detected in our Chinese blood donors was nearly 0.1%. This is 10- to 100-fold higher than the rates reported from HBV low-prevalence countries[14-17]. There are limited data about the prevalence of HBV NAT yield cases in China as detected by semi-automated in-house assays or fully automated commercial platforms[18-19]. A recent study from five geographically, ethnically, and socio-economically diverse Chinese cities reported a HBV NAT yield prevalence (0.091%) similar to our results[18]. However, Shang *et al.*[19] and Zheng *et al.*[20] reported substantially lower rates (0.0048% and 0.001%, respectively) in Shenzhen blood centre in China. A study[21] about Hong Kong donor samples reported an HBV NAT yield rate of 1 per 2,599 (0.038%) using the Novartis Procleix Ultrio assay on Tigris® for individual donations and Roche Cobas TaqScreen multiplex testing on the Cobas s201 in pools of 6. The difference in HBV NAT yield rates in different regions may be related to the use of pooled *vs* ID-NAT testing, different assays and the prevalence of HBV infection among the donors[13]. Yang *et al.*[22] reported that the yield rate of ID testing (0.21%) was 4-fold higher than that of minipools (0.05%) in Taiwanese.

It is important to recognise that Chinese blood centres do not screen blood donors for anti-HBc and our yield cases, therefore, also included OBI and window period cases. Indeed, most of the potential HBV yield cases were probable or confirmed OBI. This finding was similar to those in several other studies in which the HBV NAT cases were OBI[13,14,21-23]. In the follow-up study (donors with a negative alternative HBV NAT), three out of eight donations were still NAT-positive with the viral load less than 20 IU/mL, but HBsAg remained negative. Five of eight turned negative by the Ultrio multiplex NAT assay, which may be because of very low viral loads in the samples. Concentration of the virus in such samples would improve the rate of NAT-reactivity[24]. No NAT yield cases were found for HIV-1 or HCV; this may be related to the small number of HCV- and HIV-positive donors in this study. We found that the cost per potential HBV NAT yield case with ID-NAT was 7290 Renminbi (about 1163 US dollars). However, the blood donor screening strategy for NAT assay in mainland China should be further analysed, and the cost-efficiency ratio of ID-NAT compared to mini-pool NAT needs to be evaluated.

It is generally assumed that the level of safety of non-remunerated volunteer donations is significantly higher than that of replacement blood donations. This is supported by global data without stratifying between genuine replacement and paid donors, for first-time or repeat volunteers, or according to the age[25]. In our study, the rates of EIA-reactive/NAT-reactive samples among first-time donations were about 4-fold higher than the rates among repeat donations, indicating that repeat donations were much safer[11]. It is interesting that the rates of NAT yield cases between first-time donations and repeat donations were similar. This may be because all HBV NAT yield donations were identified at the first time they donated in the study period. Thirty-four of the 169 NAT-yield cases had donated more than five times before this study. However, we did not investigate the patients' condition after transfusion and also did not receive the HBV transfusion-transmitted disease cases report from the hospitals.

In conclusion, we determined the efficiency of NAT screening of blood donations in China and identified a higher HBV NAT yield rate. Until recently, two different EIA were required in China for screening blood donors for HBsAg, anti-HCV, and anti-HIV. However, on 1st June, 2012 a revised policy was implemented that permitted the use of a single EIA if ID/minipool-NAT for HBV, HCV and HIV had been used. Our data clearly indicate the benefit of NAT donor screening for the improvement of transfusion safety. A major benefit of NAT screening is a reduction in the risk of transmission of OBI due to the interdiction of viraemic donations that would have been missed by serological screening methods in China.

Acknowledgements

This work was supported by the Science Research Foundation of MOH (WKJ2011-2-017), and the Zhejiang Provincial Programme for the Cultivation of High-Level Innovative Health Talents. Jie Dong performed the research, analysed data and wrote the paper. Yaling Wu, Mengen Lv and Daxiao Wu performed the NAT research. Hong Zhu, Gan Li and Xiaotao Li contributed the serological studies. Faming Zhu designed the research and wrote the paper. Hangjun Lv designed the research and analysed the data.

The Authors declare no conflicts of interest.

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Arrived: 18 March 2013 - Revision accepted: 7 May 2013
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