

Significance of alanine aminotransferase testing in blood donor screening in Chongqing, China: a 7-year retrospective analysis

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ABSTRACT

Since 1993, alanine aminotransferase (ALT) testing has been mandatory for blood donor screening in China. This study aimed to evaluate the significance of ALT testing for transfusion safety. Between January 2012 and December 2018, 122 236 blood donor samples were routinely screened by the enzyme-linked immunosorbent assay method for transfusion-transmitted disease markers (TTDM) and by the kinetics method for ALT. Out of 2705 (2.21%) seropositive donors, 291 (10.76%) tested positive for ALT alone and were categorized as ALT-only positive donors. Fourteen ALT-only positive donors who all tested negative in subsequent TTDM and nucleic acid testing (NAT) screening were followed up. The return rate for ALT-only positive donors was reduced by 4.1 times as compared with qualified blood donors ($P < 0.000$). The results suggest that ALT testing does not make a significant contribution to reducing the risk of transfusion-transmitted diseases. Furthermore, being disqualified even once owing to elevated ALT levels has a significant impact on donors' return behavior. Therefore, a suitable cutoff value for ALT testing should be considered based on the evaluated risk in both blood safety and supply.

Keywords: alanine aminotransferase, serologic marker, transfusion-transmitted disease, donor return, nucleic acid test

INTRODUCTION

Alanine aminotransferase (ALT) is an enzyme that indicates cell damage in the forepart of the liver^[1–3]. Since the 1970s, ALT testing has been used in blood donor screening as a surrogate marker for viral hepatitis^[4]. However, with the application of specific serologic tests and nucleic acid tests (NATs) for detecting hepatitis B virus (HBV) and hepatitis C virus (HCV), ALT testing was discontinued for donor screening by most American and European countries

more than a decade ago^[5–8]. Nevertheless, some Asian countries, such as China and Japan, continue to include ALT testing in blood donor screening, albeit with a different cutoff value^[9–11].

China has the world's largest burden of HBV and HCV infections, with an estimated number of 86 million people having HBsAg, accounting for approximately 29% of total global infections^[12], and 9.8 million people living with chronic HCV infection, accounting for approximately 14% of total global infections^[13]. For transfusion safety, in 1993, the

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National Health Commission of the People's Republic of China issued criteria for blood donor selection which specified that blood donors must undergo ALT testing besides HBV and HCV testing. The criteria were updated three times, namely in 2012, 2015, and 2019^[9,14–15], and ALT testing was still retained in the latest criteria for blood donor selection, which was issued in October 2019. Nevertheless, because there were few studies concerning whether ALT testing helped prevent viral hepatitis transmission in China, the significance of ALT testing for transfusion safety had always been the focus of discussion by the National Health Commission of the People's Republic of China. In this survey, 122 236 individual screenings of blood donors between 2012 and 2018 in Chongqing were analyzed. This study aimed to investigate the significance of ALT testing for transfusion safety and its impact on blood donors' return behavior.

MATERIALS AND METHODS

Sample collection

This study was conducted at the Chinese PLA Blood Center in Chongqing between January 2012 and December 2018. During this period, 122 236 blood donations were screened. All donors were voluntary non-remunerated blood donors. These blood donors were classified into repeat donors who donated more than once during the observation period and first-time donors who donated only once during the observation period.

The study protocol was approved by the Medical Ethics Committee of Army Medical University (Approval No. KY2020115) and written informed consent was obtained from all donors. All experiments were performed in accordance with the relevant guidelines and regulations.

Serological tests and NATs

Blood center staff provided counseling before blood donation in the form of a questionnaire and conducted rapid testing for hemoglobin (Beijing Wangsheng Weiye Technology Development, Beijing, China), HBsAg (Asintec Technology, Xiamen, Fujian, China), and ALT testing (Acon Biotech, Hangzhou, Zhejiang, China). Blood was collected from qualified donors. The second round of ALT testing and two rounds of TTDM testing (using different enzyme-linked immunosorbent assay kits) were performed. The samples were retested if any of the screening tests yielded positive results. Finally, NATs were performed on ALT-only positive donors for HBV DNA, HIV RNA, and HCV RNA using the Procleix Tigris System (Grifols Diagnostic Solutions, San Diego, CA, USA). During the 7-year observation period, the screening strategies were the same, except for the ALT cutoff value, which was adjusted from 40 U/L to 50 U/L in 2012 (*Fig. 1*).

The second round of ALT testing was performed by clinical biochemical analysis system (AU480, Beckman Coulter, Temecula, CA, USA). Two rounds of TTDM testing were performed using the Microlab

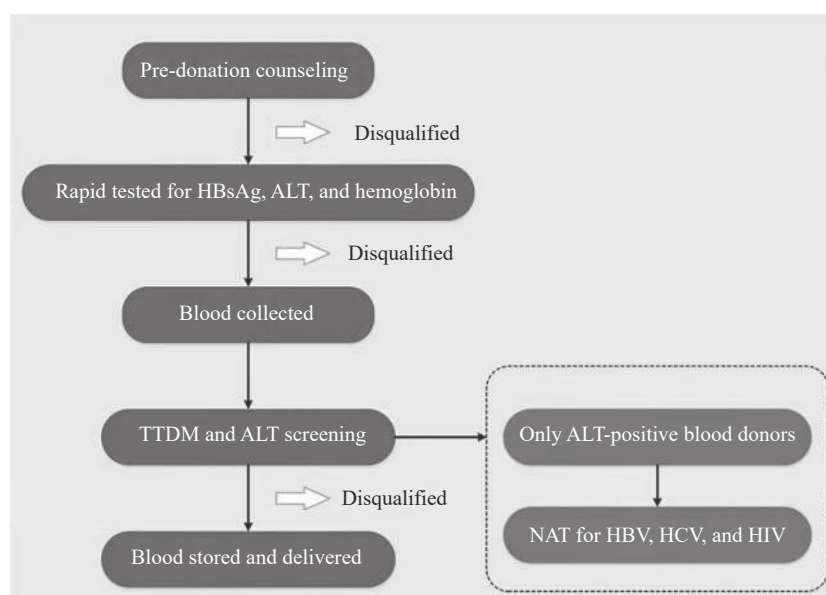


Fig. 1 Simple flow chart of donor screening

STAR and FAME system (Hamilton, Bonatus, Switzerland). HBsAg ELISA kits (Wantai BioPharm, Beijing, China; Abbott Pharmaceutical, Dartford, UK), anti-HCV ELISA kits (Asintec Technology; Abbott Pharmaceutical), HIV Ag/Ab ELISA kits (Wantai BioPharm; BIO-RAD Laboratories, Marnes-la-Coquette, France), anti-TP ELISA kits (Asintec Technology; Wantai BioPharm), and ALT kinetics kits (Beckman Coulter) were used.

Statistical analysis

Data analysis was performed using the IBM SPSS Statistics 22 software. Frequencies and cross-tabulations were used to present test positivity. The chi-square test was used to assess comparisons between groups (ALT-only positive/TTDM-positive donors). Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated for all associations. The level of statistical significance was set at $P < 0.05$.

RESULTS

Demographic characteristics of seropositive blood donors

A total of 122 236 blood donors underwent screening, comprising 77 944 (63.8%) male donors and 44 292 (36.2%) female donors. The median age of the blood donors was 27.1 years (range: 18–56 years). Among all donors, 304 (0.25%) were positive for ALT, constituting 11.24% of all 2705 (2.21%) seropositive donors. Compared with TTDM-positive blood donors, ALT-positive donors predominantly male (OR=1.43, 95% CI: 1.35–1.52, $P < 0.001$), young (OR=1.88, 95% CI: 1.43–2.46, $P < 0.001$ for donors aged 18–19 years; OR=5.59, 95% CI: 4.93–6.34, $P < 0.001$ for donors aged 20–39 years), and repeat donors (OR=401.01, 95% CI: 99.44–1617.20, $P < 0.001$) ([Table 1](#)).

Table 1 Comparisons between ALT-positive and TTDM-positive donors [n (%)]

Variables	Total (n=122 236)	ALT-positive (n=304)	TTDM-positive (n=2414)	OR (95% CI)	P
Gender					
Female	44 292 (36.2)	51 (16.8)	1011 (32.1)	Reference	
Male	77 944 (63.8)	253 (83.2)	1403 (67.9)	1.43 (1.35–1.52)	<0.001
Age (years)					
18–19	24 978 (20.4)	34 (11.2)	248 (10.3)	1.88 (1.43–2.46)	<0.001
20–39	79 930 (65.4)	229 (75.3)	1387 (57.5)	5.59 (4.93–6.34)	<0.001
40–60	17 328 (14.2)	41 (13.5)	779 (32.3)	Reference	
Donor type					
First-time	99 607 (81.5)	203 (66.8)	2412 (99.9)	Reference	
Repeat	22 629 (18.5)	101 (33.2)	2 (0.1)	401.01 (99.44–1617.2)	<0.001

ALT: alanine aminotransferase; TTDM: transfusion-transmitted disease markers (HBsAg, anti-HCV, HIV P24 antigen, anti-HIV, anti-TP); OR: odds ratio, CI: confidence interval.

Prevalence of ALT in HBV-positive and HCV-positive donors

Overall, 400 donors were found to be positive for HBV, and 67 donors were positive for HCV. Among these donors, only 0.75% of HBV-positive donors and 13.43% of HCV-positive donors exhibited elevated ALT levels ([Table 2](#)).

Table 2 Relationship between donor ALT status and HBV and HCV infections* [n (%)]

ALT status	HBV positive	HCV positive
Normal	397 (99.25)	58 (86.57)
Elevated	3 (0.75)	9 (13.43)

ALT: alanine aminotransferase; HBV: hepatitis B virus; HCV: hepatitis C virus; *: Serological and nucleic acid test results were both positive.

NAT results of ALT-only positive blood donors

During the 7-year observation period, out of the

304 ALT-positive donors, 291 were identified as ALT-only positive (TTDM-negative) donors. Among them, 190 (65.29%) were first-time blood donors, and 101 (34.71%) were repeat blood donors. All 291 blood donors tested negative in the follow-up NATs (data not shown).

Seroconversion at follow-up of ALT-only positive blood donors

Fourteen out of the 291 ALT-only positive blood donors, including 8 repeat donors and 6 first-time donors, were followed up at least once at different time intervals (range: 21–463 days). The median age of the 14 followed-up donors was 33.5 years (range: 18–51 years), and the gender ratio was 3 : 7 (male : female). Among them, 13 had normal follow-up ALT, NAT, and TTDM test results, and only one donor still had elevated ALT levels ([Table 3](#)).

Table 3 Seroconversion of ALT-only positive blood donors

Donor	Age	Gender	No. of donations	Initial screening				Followed-up screening		
				ALT	TTDM	NAT	Days	ALT	TTDM	NAT
1	28	M	9	Pos	Neg	Neg	383	Neg	Neg	Neg
2	40	M	4	Pos	Neg	Neg	276	Neg	Neg	Neg
3	43	M	11	Pos	Neg	Neg	304	Neg	Neg	Neg
4	22	M	4	Pos	Neg	Neg	21	Neg	Neg	Neg
5	36	F	3	Pos	Neg	Neg	253	Neg	Neg	Neg
6	24	M	5	Pos	Neg	Neg	462	Neg	Neg	Neg
7	35	M	3	Pos	Neg	Neg	463	Neg	Neg	Neg
8	47	F	1	Pos	Neg	Neg	194	Neg	Neg	Neg
9	19	M	1	Pos	Neg	Neg	385	Neg	Neg	Neg
10	18	M	1	Pos	Neg	Neg	454	Neg	Neg	Neg
11	47	M	2	Pos	Neg	Neg	183	Neg	Neg	Neg
12	18	M	1	Pos	Neg	Neg	370	Neg	Neg	Neg
13	41	M	1	Pos	Neg	Neg	186	Neg	Neg	Neg
14	51	F	1	Pos	Neg	Neg	234	Pos	Neg	Neg

ALT: alanine aminotransferase; TTDM: transfusion-transmitted disease markers (HBsAg, anti-HCV, HIV P24 antigen, anti-HIV, and anti-TP); NAT: nucleic acid test; M: male; F: female; Pos: positive; Neg: negative.

Return rate of qualified donors and ALT-only positive donors

Among 119 531 qualified (122 236 total, 291 ALT-only positive, and 2414 TTDM-positive) blood

donors, 23 750 (19.87%) returned to donate blood, whereas the return rate of ALT-only positive donors was 4.81% ([Table 4](#)). The return rate of ALT-only positive donors was reduced by 4.1 times as compared with qualified blood donors ($P<0.001$).

Table 4 Return rate of qualified donors and ALT-only positive donors

	Qualified donors	ALT-only positive donors	<i>P</i>
Number donated	119 531	291	
Number returned	23 750	14	
Return rate (%)	19.87	4.81	<0.001

ALT: alanine aminotransferase.

DISCUSSION

With the rapid economic development and population growth in China, the need for medical treatments is growing, leading to an increase in the demand for blood. Consequently, the current supply of blood is challenged, even though blood collection in China has been on the rise^[16]. Under the circumstances, it is critical to maintain a balance between the availability and safety of blood supply. Thus, a marker used to monitor transfusion-transmitted infection in blood donors should be chosen with caution. In China, blood donations are currently screened not only for specific transfusion-transmissible pathogenic agents but also for the non-specific marker ALT. However, most American and European countries have discontinued ALT testing for blood donors.

This study demonstrated that demographic characteristics of ALT-only positive donors were

quite different from those of TTDM-positive donors. Compared with TTDM-positive donors, ALT-only positive donors comprised higher proportions of male donors, younger donors, and repeat donors ($P<0.001$) ([Table 1](#)). Blood donors with these characteristics had a lower risk of contracting transfusion-transmitted diseases in China^[17–20]. Among hepatitis-positive donors (400 HBV positive and 67 HCV positive), only 0.75% (3/400) of HBV-positive donors and 13.43% (9/67) of HCV-positive donors had elevated ALT levels ([Table 2](#)). These results suggest that ALT testing adds little risk reduction value for transfusion-transmitted diseases, which is in line with results from previous studies in other countries^[21–24]. During the 7-year observation period, 10.76% (291 donations) of excluded blood were discarded only due to high ALT levels. Chang *et al.* reported a much higher proportion (53.0%) of excluded blood^[17], which may be due to differences in design, period, and area. In the case of blood products in short of supply, discarding such a

large amount of blood was a significant waste.

ALT is concentrated in liver tissue, and elevated ALT levels are used as a surrogate marker of liver injury. However, elevated ALT levels appear not only in pathological conditions but also in physiological conditions, such as a higher body mass index, trunk fat, visceral fat, higher hematocrit levels, and fatigue^[25–29]. In this study, 14 ALT-only positive blood donors were followed up at different time intervals, all of whom tested negatively for transfusion-transmissible pathogenic agents using ELISA and NATs. Only one case continuously had elevated ALT levels, even 234 days after donation (**Table 3**). Elevated ALT levels in blood donors were transient and non-specific, corroborating previous studies.

The return rate of ALT-positive blood donors was significantly lower than that of qualified blood donors ($P < 0.001$), indicating that ALT test disqualification has a significant impact on donors' return behavior (**Table 4**). In this study, 291 blood donors were identified as ALT-only positive blood donors; among them, 101 (34.71%) were repeat donors, with 40 (39.60%) repeat donors having donated more than four times, 11 (10.89%) more than eight times, and one more than 50 times (data not shown). Repeat donors have always been the main members of the regular donation team. However, 88 (87.1%) repeat donors stopped blood donation after being disqualified once because of one test reporting elevated ALT (data not shown).

The decision to discontinue ALT testing in China is difficult because of the higher prevalence of HBV and HCV in China compared with that in other countries. However, since the universal hepatitis B vaccination program for infants was initiated in 1992, China has made considerable progress toward controlling HBV infection^[30–32]. Furthermore, China has vigorously promoted improved hygienic habits, such as implementing the individual dining system and using serving chopsticks. Meanwhile, the latest criteria for donor screening in China were issued in October 2019, making NAT mandatory for blood donors for the first time. Hence, the residual risk of transfusion-transmitted hepatitis will be minimized.

In conclusion, ALT testing may add little risk reduction value for transfusion-transmitted diseases. However, it has a significant impact on donors' return behavior. Based on the results of this study and others, ALT testing for blood donor screening should be reassessed, and a more suitable cutoff value should be established. We hope that optimal criteria for blood donor screening will be established in China in the near future.

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