HTLV screening using chemiluminescence immunoassay among blood donors in three blood centers in China

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*Human T-cell lymphotropic virus (HTLV), blood donors, chemiluminescence immunoassay (CLIA), blood screening, prevalence*
Abstract

Background

Human T-cell lymphotropic virus (HTLV) remains a concern for blood safety. The detection of HTLV has not yet been applied for routine blood screening in China, even though lots of HTLV positive cases have been reported in southeastern China. However, data on the prevalence of HTLV infection among blood donors is limited.

Objective

The objective was to investigate the prevalence of HTLV among blood donors in three representative blood centers in China and evaluate the feasibility of chemiluminescence immunoassay (CLIA) for blood screening.

Methods

From November 2018 to March 2019, blood plasma samples collected from Hebei, Changsha and Shenzhen blood centers were screening for HTLV-1/2 antibody using Lumipulse G HTLV-I/II Immunoreaction Cartridges set (CLIA) and enzyme-linked immunosorbent assay (ELISA), followed with confirmatory test using INNO-LIA HTLV I/II.

Results

A total of 59929 blood donations were collected and screened for HTLV-1/2. The reactive rate of CLIA and ELISA among donations in Shenzhen blood center (0.0943%, 27/28621) was higher than Hebei (0.0248%, 4/16144) and Changsha (0.0198%, 3/15164) (P <0.05). After confirmation, 3 samples were confirmed as indeterminate for HTLV antibodies, only 1 sample from Shenzhen blood center was confirmed and typed as HTLV-1. The overall prevalence of HTLV-1/2 was 1.67 per 100,000 (1/59929). The HTLV-infected blood donor was a first female donor at age 32 with high school degree, born in Fujian province, and SHE minority.

Conclusion

In summary, the overall prevalence of HTLV-1/2 among blood donors in the three blood centers in China remains relatively low. However, blood donations with positive or indeterminate results for HTLV antibodies found in the study reminded us the importance of HTLV screening among blood
donors in China. In the view of HTLV prevalence and cost, all the first-blood donors should undergo HTLV screening as a systematic strategy to reduce the risk of transmission of HTLV through blood transfusion.

Introduction

Human T-cell lymphotropic virus (HTLV) discovered in the early 1980s is the first human retrovirus [1] and classified as type 1, 2, 3, and 4 [2]. HTLV is high risk factor for lymphoproliferative inflammatory and disorder, can cause adult T-cell leukemia/lymphoma (ATL) and neurological disorder [3–5]. HTLV-3 and HTLV-4 have not been reported to lead to disease [2]. It is estimated that almost 10–20 million people are infected by HTLV-1/2 in the world [6]. HTLV-1 is mainly endemic in southwestern Japan, South America, Caribbean islands, Sub-Saharan Africa, Middle East and Austro-Melanesia [7], whereas HTLV-2 infection found in Amerindian and pygmy tribes is endemic in Africa, South, North and Central America [8] The latest data described that the overall prevalence of HIV-1/2 was 2.51 per 100,000 in major areas of China, which was lower than the US, Japan and other European countries and areas [9–13]. The routes of transmission of HTLV-1/2 occur through intravenous drug use, blood transfusion and sex contact, from mother-to-child mainly through breastfeeding [14].

Several developed countries including the US, France, Netherlands, Sweden, Switzerland and Japan and et al. have conducted HTLV screening among blood donors with different screening strategies based on the prevalence of HTLV. However, the detection of HTLV-1/2 infection has not been applied for routine blood screening in China. Since 2015, according to the requirement of National Health Commission in China, blood screening laboratories only in Fujian, Zhejiang and Guangdong provinces with high prevalence of HTLV in China performed HTLV-1/2 antibody screening on all the blood donations, while only 10% donations were screened for HTLV in other provinces or municipalities. Along with the migration of population, HTLV can spread throughout China from the southeastern region (high prevalence of HTLV) [9].

Limited data about HTLV screening among blood donors in China is available. Furthermore, blood screening laboratories conduct HTLV screening using enzyme-linked immunosorbent assay (ELISA), according to the requirement of the Blood Donation Law. Automated CLIA, including sample pre-
processing system and result analysis system et al. from the same manufacturers, are fully automated and self-contained platforms which minimize operator involvement and have good reproducibility, and partly avoid the false positive/negative brought by operator factors [15, 16]. This study firstly investigated the prevalence of HTLV-1/2 among blood donors in three blood centers (South region: Shenzhen blood center, Central region: Changsha blood center, North region: Hebei blood center), using CLIA and ELISA at the same time.

Material And Method
From November 2018 to March 2019, blood donations collected from Hebei, Changsha and Shenzhen blood centers were screened for HTLV-1/2 antibody using Lumipulse G HTLV-I/II Immunoreaction Cartridges set (Fujirebio, Europe, N.V, Belgium) (CLIA) on LUMIPULSE L2400 platform (Fujirebio, Europe, N.V, Belgium). At the same time, all the donations in Changsha and Hebei blood centers underwent ELISA test using Diagnostic Kit for Antibody to HTLV-1/2 (Beijing Wantai Biological Pharmacy, Beijing, China), and blood plasma samples in Shenzhen blood center were screened by Murex HTLV I + II (Diasorin, Saluggia, Italy) in respective blood center. The sample with initial reactive results were retested twice by ELISA or CLIA using the same reagent, one of the two outputs was reactive and then identified as HTLV-1/2 reactive, followed with confirmatory test using INNO-LIA HTLV I/II (Fujirebio, Europe, N.V, Belgium), the final results were analyzed by Auto-LIA 48 automatic system (Fujirebio, Europe, N.V, Belgium). The study route and Criteria for interpreting the results obtained with the INNO-LIA strips were described in Fig. 1.

Statistical Analyses
SPSS 21.0 software was utilized for statistical analysis. Chi-square tests were performed on all examined outcomes between the tree blood screening laboratories, P < 0.05 was considered statistically significant.

Results
HTLV screening test
A total of 59929 blood donations (Hebei: 16144, Changsha: 15164, Shenzhen: 28621) were collected and screened for HTLV-1/2 using CLIA (Table 1). 115 blood donations were initially detected reactive for HTLV antibodies, of which 1 sample was only reactive for ELISA, 109 donations were only reactive
for CLIA, and 5 samples were detected as reactive by ELISA and CLIA. After repeated test, 33 blood
donations (Hebei: 4, Changsha: 3, Shenzhen: 26) were identified as HTLV reactive (Table 1). The
reactive rate of L2400 and ELISA among donations in Shenzhen blood center (0.0908%, 26/28621)
was higher than Hebei (0.0248%, 4/16144) and Changsha (0.0198%, 3/15164) (P < 0.05), whereas no
significant difference in serologic reactive rate was found between Hebei and Changsha.

Table 1
The data of HTLV-1/2 screening using CLEIA and ELISA among blood donors in the three blood screening
laboratories

| S/CO of CLEIA | Hebei blood center | Changsha blood center | Shenzhen blood center | Sum |
|---------------|-------------------|-----------------------|----------------------|-----|
|               | Initial test | Retest | Initial test | Retest | Initial test | Retest | Initial test | Retest |
| 0.1           | 2529       | /       | 2675       | /       | 2930         | /       | 8134         | /       |
| 0.2           | 6996       | /       | 6433       | /       | 12707        | /       | 26136        | /       |
| 0.3           | 4341       | /       | 4335       | /       | 8712         | /       | 17388        | /       |
| 0.4           | 1582       | /       | 1276       | /       | 2997         | /       | 5855         | /       |
| 0.5           | 490        | /       | 318        | /       | 861          | /       | 1669         | /       |
| 0.6           | 142        | /       | 88         | /       | 214          | /       | 444          | /       |
| 0.7           | 46         | /       | 27         | /       | 65           | /       | 138          | /       |
| 0.8           | 10         | /       | 5          | /       | 22           | /       | 37           | /       |
| 0.9           | 3          | /       | 3          | /       | 8            | /       | 14           | /       |
| ≥ 1.0         | 5          | 4       | 4          | 2       | 105          | 26      | 114          | 32      |
| **Number of donations** | 16144 | 15164 | 28621 | 59929 |
| **L2400 non-reactive** | 16140 (99.9752%) | 15162 (99.9868%) | 28595 (99.9092%) | 59897 (99.9466%) |
| **L2400 reactive** | 4 (0.0248%) | 2 (0.0132%) | 26 (0.0908%) | 32 (0.0534%) |
| **ELISA non-reactive** | 16144 (100.0000%) | 15162 (99.9868%) | 28617 (99.9860%) | 59923 (99.9900%) |
| **ELISA reactive** | 0 (0.0000%) | 2 (0.0132%) | 4 (0.0140%) | 6 (0.0100%) |
| **INNO-LIA negative** | 2 | 3 | 24 | 28 |
| **INNO-LIA positive** | 0 | 0 | 1 | 1 |
| **INNO-LIA indeterminate** | 2 | 0 | 1 | 3 |
| **Indeterminate rate** | 0.0124% | 0.0000% | 0.0035% | 0.0050% |
| **Negative rate** | 99.9876% | 100.0000% | 99.9930% | 99.9933% |
| **Positive rate** | 0.0000% | 0.0000% | 0.0035% | 0.0017% |

HTLV Confirmatory Test

Among 33 blood donations with reactive results for HTLV-1/2 by L2400 and/or ELISA, 29 were
confirmed as HTLV-1/2 negative, 3 were HTLV-1/2 indeterminate (Hebei: 2, Shenzhen: 1), only 1 was
positive for HTLV-1 antibodies, which was collected by Shenzhen blood center (Table 2). Among 6
samples with initial reactive results by ELISA, 1 (16.7%, 1/6) sample was confirmed as HTLV positive,
1 (16.7%, 1/6) sample was indeterminate, while 1 out of 32 samples (3.1%, 1/32) detected reactive by
CLIA was positive, and 2 samples (6.3, 2/32) with indeterminate results were observed after INNO-LIA test. Indeterminate rate of HTLV-1/2 among Hebei, Changsha and Shenzhen was 0.0124% (2/16144), 0.0000% (0/15164) and 0.0035% (1/28621) (P > 0.05). Negative rate of HTLV-1/2 in Hebei was 99.9752% (16140/16144), which was similar to Changsha (99.9868%, 15162/15164) and Shenzhen (99.9092%, 28595/28621) (P > 0.05). Furthermore, no statistical difference in HTLV positive rate was observed in the three blood screening laboratories (P > 0.05). In summary, of 59929 donations, 99.9933% (59925/59929) samples were HTLV-1/2 negative, 0.0050% (3/59929) samples were classified as indeterminate for HTLV-1/2 antibodies. The overall prevalence of HTLV-1/2 was 1.67 per 100,100 (1/59929). Demographic characteristics described that the blood donor with HTLV-1 infection in the study was a first donor and a 32-year-old woman from Fujian province and SHE minority, with high school degree.

Table 2
The details of L2400 and INNO-LIA HTLV I/II Score among blood donations with reactive results for HTLV-1/2

| Code  | ELISA | L2400 | INNO-LIA HTLV I/II Score | Result |
|-------|-------|-------|--------------------------|--------|
|       |       |       |                          |        |
| S/CO  | S/CO  | S/CO | Confirmation | Discrimination | Result |
| mean  | (Initial| (Retest) | p19 I/II | gp46 I/II | p19 I | gp46 I | gp46 II |        |
| (Retes| test) | n = 1 | p24 I/II | gp46 II | gp21 I | gp21 II |        |        |
| t)    |        | n = 2 |          |          |        |        |        |        |
| Hebei-1 | -    | 7.5  | 7.3 | 7.4 | reactiv | -   | 2+ | - | - | - | 2+ | negative |
| Hebei-2 | -    | 1.8  | 1.6 | 1.6 | reactiv | -   | - | - | 1+ | - | - | - | indeterminate |
| Hebei-3 | -    | 6.1  | 6.0 | 6.2 | reactiv | -   | - | - | - | - | - | - | negative |
| Hebei-4 | -    | 2.4  | 2.5 | 2.6 | reactiv | -   | - | 2+ | - | - | - | - | indeterminate |
| Changsha-1 | 1.8 | - | - | reactive | - | - | - | - | - | - | - | negative |
| Changsha-2 | 2.3 | 1.1 | 1.2 | 1.2 | reactive | - | - | - | - | - | - | - | negative |
| Changsha-3 | - | 2.3 | 1.2 | 1.2 | reactive | - | - | - | - | - | - | - | negative |
| Shenzhen-1 | - | 1.5 | 1.3 | 1.3 | reactive | - | - | - | - | - | - | - | negative |
| Shenzhen-2 | - | 3.8 | 3.4 | 3.4 | reactive | - | - | - | - | - | - | - | negative |
| Shenzhen-3 | - | 3.1 | 3.1 | 3.0 | reactive | - | - | - | - | - | - | - | negative |
| Shenzhen-4 | - | 1.3 | 1.4 | 1.4 | reactive | - | - | - | - | - | - | - | negative |
| Shenzhen-5 | - | 50.0| 50.0 | 50.0 | reactive | - | 1+ | - | - | 1+ | - | negative |
| Shenzhen-6 | - | 50.0| 50.0 | 50.0 | reactive | - | - | - | - | - | - | negative |
| Shenzhen-7 | - | 1.3 | 1.3 | 1.3 | reactive | - | - | - | - | - | - | - | negative |
| Shenzhen-8 | - | 1.6 | 1.6 | 1.6 | reactive | - | - | - | - | - | - | - | negative |
| Shenzhen-9 | - | 1.1 | 1.0 | 1.0 | reactive | - | - | - | - | - | - | - | negative |
| Shenzhen-10 | 1.2 | 1.2 | 1.2 | reactive | - | - | - | - | - | - | - | negative |
For the details of L2400 and INNO-LIA HTLV I/II Score among blood donations, the S/CO values of 32 blood plasma samples tested reactive, ranging from 1.0 to 5.0, occupied 84.4% (27/32), only 15.6% (5/32) samples were with S/CO value > 5.0. After confirmation, 7 samples had the confirmation lines (gag p19 I/II, gag p24 I/II, env gp46 I/II, env gp21 I/II). Of which, 3 donation with single gp46 I/II band were identified as negative for HTLV antibodies, 3 samples with single gp21 I/II were classified as indeterminate for antibodies, only 1 donation had the four confirmation lines and was confirmed as HTLV-1 positive, due to the two bands of discrimination (p19 I and gp46 I).

Discussion
Blood screening laboratories has not performed HTLV-1/2 screening on route blood donations, according to the Blood Donation Law in China. This study firstly conducted HTLV-1/2 screening among blood donors and reported the prevalence of HTLV-1/2 among blood donors in three blood centers, which were located in south, central and north region of China, respectively.

From November 2018 to March 2019, no HTLV-infected blood donors were found in Changsha and Hebei blood center, only 1 blood donors from Shenzhen blood center was confirmed as positive for HTLV-1 antibodies. The prevalence of HTLV among blood donors in Shenzhen blood center was
0.0035%, which was similar to the latest survey about the prevalence (0.0028%) of HTLV among volunteer blood donors in Shenzhen [9], while this survey found 1 blood donor with HTLV infection out of 16767 donors in Changsha blood center from January 2016 to December 2017 [9]. The small size of blood donors screened by Lumipulse G HTLV-I/II Immunoreaction Cartridges set may affect the result of epidemic investigation. At present study, the nonspecific reactive rate of CLIA in Shenzhen blood center was higher than Changsha and Hebei, which may have association with the prevalence of HTLV. According to the socio-demographic information, we found the only HTLV-infected donor in Shenzhen came from Fujian province, who was a first female donor at age 32 and SHE minority, at low educational level. Fujian province was the area with the highest prevalence of HTLV in China [17]. Shenzhen is the city with the largest population flow and included in Guangdong province, which is the second HTLV epidemic area in China [17]. Hence, Shenzhen may have more blood donors with HTLV infections, compared with Changsha and Hebei.

Although 1 sample was confirmed as HTLV-1 positive, 6 donations had the confirmation lines in INNO-LIA HTLV I/II Score. Of these, 3 samples were indeterminate, due to the high possibility of HTLV antibodies. An indeterminate result on the INNO-LIA HTLV I/II Score needs follow up, and additional testing such as PCR is recommended by the manufacturer. Among 32 samples tested reactive by CLIA, the majority of samples were with S/CO value 1.0–5.0, three samples were strongly reactive for HTLV-1/2 by CLIA (S/CO ≥ 50), but two samples were detected non-reactive by ELISA, and identified as negative for HTLV-1/2 antibodies after INNO-LIA test, the mutations in testing targets may resulted in the missing of INNO-LIA. Furthermore, 1 sample with indeterminate result by INNO-LIA tested reactive by CLIA but was missed by ELISA, even though ELISA had higher specificity than CLIA in this study, which reflected that CLIA may have higher sensitivity than ELISA [18].

In summary, the overall prevalence of HTLV-1/2 among blood donors in the three blood centers in China was 0.0017%, which was lower than Japan, the US and many European countries that have implemented HTLV donor testing. However, blood donations with positive or indeterminate results for HTLV antibodies found in the study reminded us the importance of HTLV screening among blood donors in China. CLIA with high sensitivity can be used for routine blood screening [18]. Due to the
high cost of CLIA and INNO-LIA, HTLV screening among blood donors with CLIA did not last for a long time, limited sample size may bias the epidemiological results, and all the donations with non-reactive results by CLIA and ELISA were not further confirmed by INNO-LIA, which may lead to the missing of HTLV-infected blood donors. In the view of HTLV prevalence and cost, we supported the view that all the first-blood donors should undergo HTLV screening as a systematic strategy. Moreover, blood screening laboratories in this study remain relatively few. Future research must focus on expanded sample size and geographical coverage to get a more comprehensive dataset about HTLV prevalence to improve blood safety.

Declarations

Data Availability
The data used to support this study are available from the corresponding author upon request.

Ethics approval
The institutional review boards of the Shenzhen, Changsha and Hebei blood centers respectively have approved the study. The methods in the study were in accordance with the guidelines of the Declaration of Helsinki. Written informed consent was obtained from all subjects participating in this research.

Conflicts of Interest
The authors declare that they have no conflicts of interest.

Authors’ contribution
Junpeng Zhao and Jinfeng Zeng designed the study. Junpeng Zhao, Feixue Zhao, Wei Han and et al. conducted the laboratory tests. Junpeng Zhao and Xiaoxuan Xun collected and analyzed data and prepared the manuscript. Junpeng Zhao and Jinfeng Zeng edited and reviewed the manuscript. All Authors critically reviewed and revised the manuscript drafts, approved the final version of the manuscript and take responsibility for the integrity of the data and accuracy of data analysis.

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References
1. Poiesz BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD, Gallo RC: Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proceedings of the National Academy of Sciences of the United States of America 1980, 77(12):7415-7419.

2. Mahieux R, Gessain A: HTLV-3/STLV-3 and HTLV-4 viruses: discovery, epidemiology, serology and molecular aspects. Viruses 2011, 3(7):1074-1090.

3. Osame M, Usuku K, Izumo S, Ijichi N, Amitani H, Igata A, Matsumoto M, Tara M: HTLV-I associated myelopathy, a new clinical entity. Lancet (London, England) 1986, 1(8488):1031-1032.

4. Gessain A, Jouannelle A, Escarmant P, Calender A, Schaffar-Deshayes L, de-The G: HTLV antibodies in patients with non-Hodgkin lymphomas in Martinique. Lancet (London, England) 1984, 1(8387):1183-1184.

5. Hinuma Y, Nagata K, Hanaoka M, Nakai M, Matsumoto T, Kinoshita KI, Shirakawa S, Miyoshi I: Adult T-cell leukemia: antigen in an ATL cell line and detection of antibodies to the antigen in human sera. Proceedings of the National Academy of Sciences of the United States of America 1981, 78(10):6476-6480.

6. Willems L, Hasegawa H, Accolla R, Bangham C, Bazarbachi A, Bertazzoni U, Carneiro-Proietti ABdF, Cheng H, Chieco-Bianchi L, Ciminale V et al: Reducing the global burden of HTLV-1 infection: An agenda for research and action. Antiviral Res 2017, 137:41-48.

7. Gessain A, Cassar O: Epidemiological Aspects and World Distribution of HTLV-1 Infection. Frontiers in microbiology 2012, 3:388.

8. Roucoux DF, Murphy EL: The epidemiology and disease outcomes of human T-lymphotropic virus type II. AIDS reviews 2004, 6(3):144-154.

9. Li L, Ou S, Huang C, Zhou X, Ge H, Li J, Zeng J, Zhou A, He L, Xu Q et al: The
prevalence of human T-cell leukemia virus in blood donors in China.

Transfusion 2019, 59(7):2361-2367.

10. Satake M, Yamaguchi K, Tadokoro K: Current prevalence of HTLV-1 in Japan as determined by screening of blood donors. Journal of medical virology 2012, 84(2):327-335.

11. Pillonel J, Le Marrec N, Girault A, David D, Laperche S: Epidemiological surveillance of blood donors and residual risk of blood-borne infections in France, 2001 to 2003. Transfusion clinique et biologique : journal de la Societe francaise de transfusion sanguine 2005, 12(3):239-246.

12. Laperche S, Worms B, Pillonel J: Blood safety strategies for human T-cell lymphototropic virus in Europe. Vox sanguinis 2009, 96(2):104-110.

13. Malm K, Ekermo B, Hillgren K, Britton S, Fredlund H, Andersson S: Prevalence of human T-lymphotropic virus type 1 and 2 infection in Sweden. Scandinavian journal of infectious diseases 2012, 44(11):852-859.

14. Pereira FM, de Almeida M, Santos FLN, Carreiro RP, Regis-Silva CG, Galvao-Castro B, Grassi MFR: Evidence of New Endemic Clusters of Human T-Cell Leukemia Virus (HTLV) Infection in Bahia, Brazil. Frontiers in microbiology 2019, 10:1002.

15. Sasano M, Kimura S, Maeda I, Hidaka Y: Analytical performance evaluation of the Elecsys(R) Cyclosporine and Elecsys(R) Tacrolimus assays on the cobas e411 analyzer. Pract Lab Med 2017, 8:10-17.

16. Sommese L, Sabia C, Paolillo R, Parente D, Capuano M, Iannone C, Cavalca F, Schiano C, Vasco M, De Pascale MR et al: Screening tests for hepatitis B virus, hepatitis C virus, and human immunodeficiency virus in blood donors: evaluation of two chemiluminescent immunoassay systems. Scandinavian journal of infectious diseases 2014, 46(9):660-664.
17. Du J, Chen C, Gao J, Xie J, Rong X, Xu X, Wang Y, Wang F, Li J, Lu Z et al: **History and update of HTLV infection in China.** *Virus Res* 2014, **191**:134-137.

18. Berini CA, Susana Pascuccio M, Bautista CT, Gendler SA, Eirin ME, Rodriguez C, Pando MA, Biglione MM: **Comparison of four commercial screening assays for the diagnosis of human T-cell lymphotropic virus types 1 and 2.** *Journal of virological methods* 2008, **147**(2):322-327.

**Figures**

**Figure 1**

The study routes of HTLV screening test and confirmatory test.