Transfusion and Apheresis Science 43 (2010) 269–272

Contents lists available at ScienceDirect

Transfusion and Apheresis Science

journal homepage: www.elsevier.com/locate/transci

Can the nucleic acid amplification test (NAT) be an alternative to the serologic tests? A prospective study, the results of 18,200 blood donors from the Turkish Red Crescent ☆

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ARTICLE INFO

Keywords:
HIV
HBV
HCV
Nucleic Acid Amplification Test (NAT)

ABSTRACT

Aim: Serologic tests having high sensitivity and specificity are used in order to prevent contamination with infectious agents from blood and blood products for transfusion safety. The present serologic tests have problems such as low sensitivity and weak detection capacity of infectious agents in the "window period". We aimed to test the use of NAT (Nucleic Acid Amplification Test) in routine blood screening in the Blood Bank.

Method: We used the Procleix Ultrio (Chiron Ltd., USA) test kit based TMA (Transcription Mediated Amplification) for the NAT study of serum samples from 18,200 donors who came to the Turkish Red Crescent between February 2007 and September 2008. The NAT positive samples were studied twice. The discrimination of HIV, HCV and HBV NAT positive samples was performed by the Procleix Discrimination (Chiron Ltd., USA) test. Otherwise, Micro ELISA were used in parallel for routine serological screening of Anti-HIV, Anti-HCV, and HBsAg with Vironoste HIV Uni-form, AG/Ab innotest HCV Ab and Hepanostika Ultra HBsAg test kits.

Results: The results of serum samples with serology (+) and NAT (+) (13/18,200 and 0.05%) for anti-HIV, anti-HCV and HBsAg were higher than in other NAT studies; we also detected that a transfusion risk can occur in every 1400 transfusions.

1. Introduction

High sensitivity serologic tests are generally used in blood banks to prevent and reduce the transmission risk of infectious agents in blood and blood products; however, it is nearly impossible to eliminate the risk of transmission completely due to problems with the sensitivity and specificity of existing serologic methods and the window period following a recent, undetected viral infection. For these reasons, NAT has recently become the center of attention as an alternative routine blood screening method, with the goal of obtaining100 percent safety against viral infection of blood during transfusions [1–3].

In this study, we compared NAT and routine serological test results from 18,200 individuals, who donated blood at

☆ This study was presented in 19th ECCMID, Helsinki, Finland, May 16–19, 2010.

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doi:10.1016/j.transci.2010.09.013

the Turkish Red Crescent Blood Center, and investigated if NAT could be used as a routine screening test for HIV, HBV, and HCV.

2. Materials and methods

2.1. Study group

This work was conducted as a cross-sectional study at the Turkish Red Crescent Çapa Blood Center of Istanbul intermittently from February 2007 to March 2008. Of the total 18,200 voluntary blood donors 546 (3%) were women, 17,654 (97%) were men, 2 (0.1%) were regular donors, and 18,198 (99.9%) were first time donors. The donors were between 18 and 60 years old; the mean age was 40.

2.2. Sample collection and methods

2.2.1. Sample collection

Applications from 18,200 volunteers were first received by the blood center, and the individuals who completed their physical exams were accepted as donors. Ten milliliter samples of non-coagulated blood were collected from each donor to test for anti-HIV/1–2, anti-HCV, HBsAg and TPHA. Serum samples were obtained after centrifugation of blood at 3000 rpm. For the NAT study, a second set of blood samples were collected from the same individuals into 9 ml sterile tubes containing anticoagulant and ethylenediamine tetra-acetic acid (EDTA). Serum was similarly obtained from these blood samples by centrifugation at 3000 rpm.

2.2.2. Serological study methods

Each serum sample was tested for anti-HIV/1–2, anti-HCV, HBsAg and TPHA. First, three tests were done using Vironostika HIV Uni-Form II Ag/Ab (bioMerieux Inc., France), Innotest HCV Ab III (Innogenetics NV, Belgium), and Hepanostika Ultra HBsAg (bioMerieux Inc., France) kits, respectively, on a Da-Vinci automatic micro-ELISA instrument (bioMerieux Inc., France); the TPHA test was performed last using a THPA kit (Randox Laboratories Ltd., USA). Furthermore, anti-HBc and anti-HBe tests were conducted in serologically negative, but NAT test positive samples, using a Hepanostika HBc Uniform Total test kit (bioMerieux Inc., France) and anti-HBs kit (Biokit S.A., Spain), respectively.

The samples were studied after the sera were found to be reactive during the serological study. The serum was accepted as reactive when two out of three tests were positive. In addition, an immuno-blot test was done with an Innolia kit (Innogenetics NV, Belgium) to confirm the anti-HIV and anti-HCV tests. For the serologically negative but NAT positive serum samples, a micro-ELISA was performed using the macro-ELISA Axim instrument (Beckman-Coulter, Inc., USA) with Axim HIV Ag/Ab Combo, Axim HCV Version III, and Axim HBsAg 2.0 (Abbot Laboratories, USA) kits to confirm negativity; the results were ensured to be negative using an Access instrument (Beckman-Coulter, Inc., USA) with Beckman-Coulter Access HIV/1–2 Ab (Beckman-Coulter, Inc., USA), Beckman-Coulter Access HCV Ab (Beckman-Coulter, Inc., USA), and Access HBsAg (Beckman-Coulter, Inc., USA) kits.

2.2.3. NAT studies

In the NAT studies, the Procleix Ultrio kit (Chiron, USA) was used with the TMA (Transcription-Mediated Amplification) method based on RNA transcription amplification which targets both RNA and DNA with RNA polymerase and reverse transcriptase enzymes; 100–1000 copies/ml were amplified in each cycle using the Chiron Tigris automatic system (Chiron, USA). The Procleix Discrimination test kit (Chiron, USA) HBV was used for discrimination of HBV, HCV and HIV/1.

The same serum sample was studied two more times after it was determined to be positive with the Procleix Ultrio kit, and when it was found to be positive in at least one of the two studies, it was confirmed to be reactive according to the NAT method. The sample was studied with Procleix Discrimination HBV, HCV, and HIV/1 kits, respectively. The virus was detected when it produced a positive result; however, the sample that was found positive in the first NAT assay, but negative in the following two NAT assays, was considered to be a false-positive.

3. Results

During this study 18,200 donors were tested using serological methods. Of these donors 24 (0.13%) were anti-HIV/1–2 positive, 66 (0.36%) were anti-HCV positive, and 318 (1.75%) were HBsAg positive. According to the NAT discrimination test 4 (0.02%) donors were HIV/1 positive, 12 (0.06%) were HCV positive and 312 (1.72%) were HBV-DNA positive (Table 1). When these results were evaluated, according to a serology (−)/NAT (+) combination, 2 donors were HCV positive, 11 were HBV positive and negative by serology, and 37 donors were false positive, according to NAT. When the results were evaluated, using the serology (+)/NAT (+) combination, 9 donors were found HCV positive, 297 were HBV positive, 3 were HIV positive, 3 were both HBV and HCV positive, and 1 donor was both HBV and HIV. The combination of the other results is shown in Table 2. Two HCV infected patients tested as serology negative–NAT positive and had anti-HBc depending on HBV positivity; anti-HBc was positive in a total of 11 HBV infected cases, while in 5 cases both anti-HBc and anti-HBs were positive. However, HBV was positive in 297 cases using both serology and NAT/Discrimination tests, 297 of these were anti-HBc positive and 6 were detected to have both anti-HBc and anti-HBs (Table 3).

4. Discussion

Maintaining a safe blood supply constitutes an important challenge for today's blood banking system, as the

Table 1
Serology and NAT results of 18,200 donors.

Donors	Anti-HIV/HIV RNA	Anti-HCV/HCV RNA	HBsAG/HBV DNA
18,200	24/4	66/12	318/312

Table 2
HCV, HIV and HBV results due to Serology and NAT patterns.

Serology and NAT patterns	HCV	HIV 1	HBV	HBV + HCV	HCV + HIV	HBV + HIV
Serology (+) NAT (−)	56	19	17		1	
Serology (−) NAT (+)	2 (1:9100)	–	11 (1:1654)			
Serology (+) NAT (+)	9	3	297	3		1
Serology (−) NAT (−)			17,784			
Serology (−) NAT:FP*			37			

* FP, False positivity.

Table 3
Seroloji ve NAT test birlikteliğine göre anti-HBc and anti-HBs results due to Serology and NAT patterns.

Serology patterns	Serology and NAT patterns		
	S*(−)/NATD**(+) S(+)/N:D(+) HCV (n:2)	HBV (n:11)	HBV (n:297)
Anti-HBc	2	11	297
Anti-HBc/anti-HBs	–	5	6

* S, serology.
** NATD, NAT discrimination.

consequences of an unsafe blood supply pose a very serious public health problem. The World Health Organization (WHO) describes safe blood as 'blood that does not contain any infectious agents, drugs, alcohol, chemical substances, or other extraneous factors that might cause harm, danger or disease to the recipient'. Although one of the most important parameters for safe blood is for the blood to be non-infectious, it seems impossible to achieve zero risk of infection because of the transmission ratio of infectious agents by the conventional screening methods that are currently available. Although the risk of infection via blood and blood products has decreased significantly over the years, it has not been completely eliminated [2]. The American Association of Blood Banks (AABB) announced that the number of post-transfusion hepatitis cases was 4987 between 1981 and 1994. Of these cases 24 percent were HBV positive and 43 percent were HCV positive. The Center for Disease Control and Prevention (CDC) also announced that the number of AIDS cases reached 7,93,026 in July, 2001 in the USA. Of these AIDS cases 9276 (1.2%) were infected by transfusion [4,5]. In socio-economically underdeveloped countries, such as the Congo, the infection rate due to transfusion was 40 percent in the 25 percent of paediatric HIV cases aged >1. At the end of 2006, infection by transfusion was 43 (1.78%) of the total 2412 seropositive + AIDS in Turkey [6,7]. In addition to this data, the German Red Cross (GRC) blood donor services claimed that 121 of the total of 100,000 anti-HCV negative blood samples caused infections despite all the screenings efforts; in the USA the infection risk was reported as 1/676,000 for HIV, 1/63,000 for HBV, and 1/103 for HCV [8,9]. A residual risk is mainly due to donations of blood during the window period, virus variants, atypical seroconversion (immunosilent period) and laboratory errors at a rate of 0.1% [9]; among these it was reported that the window period was the most important problem. The window period for HBV is 56 days, it is approximately 70 days for HCV (via third generation ELISA kits) and an average of 16–17 days for HIV (by p24 antigens) [10]. Therefore, to minimize the risk of infections through blood transfusion and blood products, the focus was on the efficacy in nucleic acid based methods for viral agents, especially, HBV and HCV in blood banking. Recently, in developing countries, mathematical models based on nucleic acid test (NAT) methods have been developed to detect the size of a viral infection. According to these models it is estimated that the overall viral risk is 1/100,000–1/500,000 before NAT and <1/1000,000 after NAT [11].

Hourfer et al. reported that 9 HCV, 3 HIV/1 and 9 HBV positives were detected during their NAT study of 9,549,216 donors, suggesting that the estimated infection risks are 1 per 6.2 million for HCV and 1 per 3.6 million for HIV/1 [12]. According to Schellenberg et al. among the 1.4 million voluntary, non-remunerated blood donors, 15 donations tested positive for HIV, 119 for HCV and 218 for HBV. Among these, 5 first time donors were positive for HIV, 103 for HCV and 218 for HBV. In contrast, it was reported that 10 repeat donors seroconverted for HIV, 16 for HCV and 11 for HBV. In their report the prevalence and incidences of the transfusion relevant viruses HCV, HIV and HBV were considerably lower after repeated donations. Thus, confirmed positive blood donations due to seroconversion were 66 times lower for HCV and 188 times lower for HBV when compared to the prevalence in first time donors [13]. Kalibatas et al. [14] screened a total of 59,432 donations using an NAT method similar to one that was used in our work; according to that study 15 HCV cases and 5 HBV cases were detected as positive by NAT in 59,432 serology negative donors.

Seifried et al. [15] screened for 16,272,434 blood donors between 1997 and 2003 using samples with pool sizes of 48 and 96. They screened 18,345,372 blood samples for HCV, 16,367,514 for HIV and HBsAg; they detected 13 HCV, 38 HBV and 3 HIV positive cases by NAT. In our study group, consisting of 18,200 first time donors and 2 repeat donors, 11 HBV cases and 2 HCV cases were detected as serology negative but NAT positive; none of them were positive for HIV. Our NAT study result (13/18,200: 0.05%) was higher than those of the other studies; the viral infection risk was 1 per 9100 transfusions for HCV and 1 per 1654 transfusions for HBV, the total being 1 per 1400 transfusions for HBV and HCV. Besides, serologic-negative and NAT positive, 11 of 11 HBV cases were found to be anti-HBc positive, in 5 cases anti-HBc and anti-HBs were

detected with low levels of viremia titres (between 11 and 69 IU/ml).

In this study, we conclude that the number of serologic-negative and NAT positive donors was higher than those of other studies and a viral risk (HBV, HCV) was found in 1 of 1400 transfusions. All these were due to a high percentage of first time blood donors, since nearly all were first time donors. If we consider the high costs of NAT testing compared with other serologic tests, NAT was not found to be efficient in all studies on a cost-based effectiveness. Therefore, NAT should be applicable only for first time donors at regional blood centers. We conclude that gaining more regular blood donors would be the most beneficial solution for blood centers.

5. Conflicts of interest

On behalf of all authors, as the corresponding author Bekir Kocazeybek, I confirm that there are no financial or personal relationships with other people or organisations that could inappropriately influence (bias) our work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding.

6. Role of the funding source

There was no role for sponsors in our study.This work has been approved by the Cerrahpasa Medical Faculty ethical committees.

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