Evaluation of serological transfusion-transmitted viral diseases and multiplex nucleic acid testing in Malaysian blood donors

Saif Ghazi Yaseen a,⇑, S.A. Ahmed b, M.F. Johan b, R. Kiron c, Aqil Mohammad Daher d

a Department of Pathology/Haematology Unit, Universiti Teknologi MARA, Malaysia
b Departments of Haematology and Transfusion Medicine Unit, School of Medical Sciences, Universiti Sains Malaysia, Malaysia
c Synapse Sdn Bhd, Kelana Jaya Petaling Jaya 47301, Malaysia
d Population Health and Preventive Medicine, Faculty of Medicine, Universiti Teknologi MARA, Malaysia

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ABSTRACT

Background: Transmission of infectious diseases is a recognized complication of blood transfusion and blood products. Nucleic acid testing (NAT) may contribute to improved efficiency of blood screening and thereby increase the safety margin for transfused blood. Methods: Unscreened blood samples from 1388 randomized donors were selected for this study at the Transfusion Medicine Unit of Hospital Universiti Sains Malaysia (HUSM). Informed consent was obtained from all donors and blood samples were tested for HIV, HBV and HCV serologically and by NAT assay. Results: Of the 1,388 tested samples, 1,360 were non-reactive for both assays. Four samples (0.29%) were both serologically and NAT reactive. The remaining twenty-four samples were divided into two groups. Of these, five samples (0.366%) were NAT reactive and nineteen samples (1.37%) were serologically reactive. However, serology confirmation tests run on the latter nineteen samples were non-reactive. Conclusions: Hence, NAT adds benefit of detecting “false positive” reactions via standard serology, the cost of administering NAT also needs further consideration and study.

1. Introduction

The supply of safe and efficacious blood and blood components for transfusion or manufacturing involves multiple steps beginning with (1) the selection of blood donors; (2) the collection, processing and testing of blood donors and testing of patient samples; (3) and the issue of compatible blood recipients and its administration. Each step of the “transfusion chain” holds risks of error and failure that have serious implications for recipients of blood and blood products [1]. All donors in Malaysia are unpaid volunteers who are carefully selected by using a donor health questionnaire to ensure safety and reduce the risk of transmitting infectious vectors. In Malaysia as well as in many other countries, blood samples are routinely serologically screened for Hepatitis B virus (HBV), human Immunodeficiency Virus Type 1 (HIV-1), and Hepatitis C Virus (HCV). Transmission of HIV, HCV and HBV by blood and blood components has remarkably declined over the past two decades as a result of implementing sensitive and specific tests for the detection of viral antibodies and antigens [2,3]. Hence, the residual risk of transfusion-transmitted viral infection is considered minimal or negligible. However, attaining ‘zero risk’ remains a substantial challenge [1,4]. Genomic screening for HBV, HCV and HIV represents a major advance that can eliminate infectious blood donations collected during the pre-seroconversion window period, meaning donations can occur when the donor is infectious but nonreactive to assays for viral antibodies or antigens [3]. The seronegative window phase for
Hepatitis C is estimated at an average of eighty-two days for second-generation enzyme immunoassays (EIA) [5], but was reduced to approximately sixty-six days for third generation EIA [6–8]. Hepatitis B and HIV have window phases of approximately fifty-nine and twenty-two days, respectively [5]. Cases of viral infection have been documented in patients who received blood or blood components from donors during the window phase of infection [9,10]. Other sources of residual risk are rare cases of ‘immunosilent’ infections, which possibly include a large spectrum of viral variants that are not detectable when using tests designed to identify their common forms. These include: atypical seroconversion as indicated by an unusually prolonged window period or lack of seroconversion, as well as emerging viral mutants. Laboratory error is still another source of residual risk [3,11,12]. The recent introduction of Nucleic Acid Testing (NAT) as a screening tool for blood donors may enhance the safety of the blood supply. Thus, donors that otherwise escape detection by routine serology during an ‘infectious’ window period may be identified via NAT assay [1]. Presently, NAT testing is recommended for government and private organizations in pursuit of providing “zero risk – safe blood” to recipients. Nevertheless, the necessity and feasibility of achieving “zero-risk” for blood transmissible viral agents is currently questioned, especially in view of high cost vs. benefit of the additional measure [1,4,13].

2. Materials and methods

This cross sectional prospective study was conducted over a period of fourteen months from November 2008 through January 2010 at the Transfusion Medicine Unit of Hospital Universiti Sains Malaysia (HUSM). A cohort of 1,388 donors who fulfilled the eligibility criteria for blood donation was selected by a systematic random sampling method applied to registered donors. We estimated a need for five donors daily given an estimated attendance at the blood bank of approximately fifty donors per day. The sampling interval was fixed at every tenth patient and ceased after five samples were taken daily.

This study was approved by the School of Medical Science Research and Ethics Committee. Written informed consent was obtained from all donors after the nature of the study was fully explained.

The NAT assays were conducted at the Transfusion Medicine Unit, HUSM, utilizing the individual test (ID) NAT Assay (Chiron Corporation, Emeryville, CA) according to the manufacturer’s instructions. Each sample was tested individually by NAT and concordantly with ELISA. The ID NAT assay is a multiplex test that provides simultaneous detection of HIV-1 RNA, HCV RNA and HBV DNA in human plasma by using transcription mediated amplification technology (TMA).

When a tested sample was reactive by NAT and ELISA, all blood bags were discarded. Any sample with discordant results was followed up prospectively during the next visit with further analysis as follows: Blood was taken directly from the donor and sent for a second run for ELISA confirmation. In cases where the NAT was initially reactive, donor samples were tested in duplicate using the same NAT kit prior to the discriminatory assay. If the ELISA was initially positive, the donor’s blood bag was discarded and a second sample was taken directly from the donor (by bleeding) for a second ELISA assay and confirmatory serological testing.

The confirmatory serology test was performed in the microbiology laboratory unit of HUSM in order to identify false positive reactivity during serological screening as it is more sensitive and specific. We performed neutralization tests for HBsAg, INNO-LIA HCV Score for HCV, and particle agglutination for HIV.

The INNO-LIA HCV Score is a Line Immuno Assay (LIA) for the detection of antibodies to Human Hepatitis C Virus in human serum or plasma. It is intended for use as a supplementary test of human serum or plasma specimens found reactive using the anti-HCV screening method.

The discriminatory assay/test was performed when an individual test was initially reactive. The HIV-1, HCV, and HBV discriminatory assays utilize the same three steps taken for the ID NAT Assay (target capture, TMA and HPA). The same assay procedure was followed with one major difference: HIV-1-specific, HCV-specific, and/or HBV-specific probe reagents were used in place of the ID NAT assay probe reagent.

3. Results

Characteristic profiles of the study’s cohort are shown in Table 1. The age of donors ranged from eighteen to sixty years of age with mean of 28.9 years (Standard Deviation: 10.95). The majority were Malays (85.37%), followed by Chinese (12.96%), Indian (1.15%), and other races (0.5%). Altogether there were 376 women (27.08%), and 1,012 men (72.91%).

Of the 1388 donor samples studied, the prevalence for ‘window case’ HIV-1, HCV and HBV in HUSM using NAT was 0.65% (nine cases) which required another procedure to identify the type of virus via discriminatory testing. However, this prevalence was reduced to less than half after the discriminatory test was performed as there were only four positive cases identified (HBV DNA) yielding a prevalence of 0.29% (see: Table 2).

In terms of standard serology technique, twenty-three cases were identified as positive via serological reaction giving a prevalence of 1.66%. Subsequently, nineteen of the twenty-three cases were found negative via serological confirmatory testing, reducing the prevalence to 0.29%. The
prevalence rates for viral detection using standard serological screening tests were for 0.14% for anti-HIV (two cases); 0.29% HBsAg (four cases); and 1.22% for anti-HCV (seven-teen cases) (see: Table 3).

Four cases of HBV were detected by both NAT and serology (17.40%). All four cases were reactive by confirmatory (neutralization test for HBsAg) and discriminatory tests and were therefore not considered ‘window’ cases. The five cases (0.40%) identified as reactive by ID NAT were negative via serology totaling nineteen samples (82.60%) that were serologically reactive but NAT nonreactive. Therefore, this study demonstrated that NAT sensitivity, as measured against serological screening, was 17.40%, whereas the specificity of NAT measure against serological screening was 99.6% (see: Table 4).

However, the final comparison between NAT and serological confirmatory testing for selected reactive cases appreciably changed NAT’s sensitivity from 17.3% to 100% when all nineteen samples were deemed negative by confirmatory test, which established their false positive status as per initial serological screening. NAT specificity was also reduced to 79.2% when compared with confirmatory serological tests that yielded five confirmed, nonreactive samples (see: Table 5).

4. Discussion

Viral detection among blood donors is crucial to the prevention of transfusion related infections. Most studies on the detection of ‘residual yield’ cases are of molecular testing and come from developed countries. To the best of our knowledge we are reporting the first study utilizing a genomic technique for donor screening in Northern Malaysia. These findings are expected to add to the growing evidence that supports benefits derived from the use of genomic detection of viruses for the early detection of window cases; specifically, the reduction of infectious transmission risks in blood and blood products.

Nevertheless, this study strongly suggests that the NAT may prevent exceedingly rare cases of viral transmission indicating it is not necessarily warranted or justified as the primary test of choice. Nonetheless, from data provided by the National Blood Bank Center of Malaysia, the NAT yield in Malaysia, when compared to other countries, is high. In countries like Singapore, Thailand, Hong Kong, Korea, India and Malaysia, NAT yields had been reported as 1:24,567, 1:11,676, 1:202,500, 1:146,628, 1:2,666, 1:3,286, respectively [14].

The five NAT (ID) reactive cases in this study were confirmed as non-reactive via serology and discriminatory testing. Hence, NAT was regarded as yielding positive but indeterminate results. The possibility that undergirds this discrepancy is that NAT is likely to yield window cases with low viral loads that may at times go unrecognized by discriminatory assays [15]. The discrepancies between ID NAT and discriminatory testing may be attributed to the low viral content of the samples tested rather than false positive results or decreased sensitivity of discriminatory assay [15]. Charlewood and Flanagan stated that the multiplex assay is more sensitive than the discriminatory assay. However, this is denied by the assays’ package inserts and by Gen-Prob (Jeffrey Linnen, personal communication) [16].

In addition, other causes of indeterminate results include occult hepatitis B infection (OBI); a low level HBsAg carrier; mutation at the HBsAg region; insensitivity of the HBsAg assay; technical errors; and false positive result. The cited discrepancy is a major problematic issue because false reactivity cannot be distinguished from the possibility of an occult infection without screening blood donors for anti-HBc routinely [16]. Furthermore, Occult HBV in blood donors has a wide range of potential etiologies within the natural history of the infection. It may originate from (a) recovered infections with anti-HBs and persistent, low-level, viral replication; (b) escaped mutants that are undetected by HBsAg assays; or (c) a healthy chronic carrier—the latter being found mostly with anti-HBc. In addition, antibody markers may become undetectable which over time leaveing HBV DNA as the only marker of infection.

Table 2

<table>
<thead>
<tr>
<th>NAT reaction</th>
<th>Count</th>
<th>%</th>
<th>Discriminatory NAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not reactive</td>
<td>1379</td>
<td>99.35</td>
<td>Negative 1384 99.71%</td>
</tr>
<tr>
<td>Reactive</td>
<td>4</td>
<td>0.29</td>
<td>Positive 4 0.29%</td>
</tr>
<tr>
<td>Reactive</td>
<td>5</td>
<td>0.36</td>
<td></td>
</tr>
</tbody>
</table>

Table 3

<table>
<thead>
<tr>
<th>Serology reaction</th>
<th>Serology confirmatory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not reactive</td>
<td>1365 98.34% NEGATIVE 1384 99.71%</td>
</tr>
<tr>
<td>HIV</td>
<td>2 0.14% POSITIVE 4 0.29%</td>
</tr>
<tr>
<td>HBV</td>
<td>4 0.29%</td>
</tr>
<tr>
<td>HCV</td>
<td>17 1.22%</td>
</tr>
</tbody>
</table>

Table 4

<table>
<thead>
<tr>
<th>NAT reactive</th>
<th>Sero-reactivity</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative%</td>
<td>Positive%</td>
</tr>
<tr>
<td>Not reactive</td>
<td>1360 99.60%</td>
<td>19 99.40%</td>
</tr>
<tr>
<td>Reactive</td>
<td>5 4 23 1388</td>
<td>9 0.60%</td>
</tr>
<tr>
<td>Total</td>
<td>1365 23 1388</td>
<td>0.40% 0.60%</td>
</tr>
</tbody>
</table>

Table 5

<table>
<thead>
<tr>
<th>NAT reactive</th>
<th>Serology (confirmatory)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative%</td>
<td>Positive%</td>
</tr>
<tr>
<td>Not reactive</td>
<td>19 79.20%</td>
<td>0.00%</td>
</tr>
<tr>
<td>Reactive</td>
<td>5 20.80%</td>
<td>100.00%</td>
</tr>
<tr>
<td>Total</td>
<td>24 100.00%</td>
<td>100.00%</td>
</tr>
</tbody>
</table>

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In all instances, the viral load is low, mostly $<10^4$ IU/ml and often $<100$ IU/ml. At such low levels, nucleic acid testing (NAT) in pools is likely to be largely ineffective [17,18]. Therefore, an initial reactive result showing non-discriminate results should not be considered a false reactive until proven otherwise and caution should be taken as to whether to accept or defer such donors [16].

Expected findings from this study included serology and ID NAT reactive discrepancies. We found that the majority of discrepant reactive results reacted with anti-HCV antibodies but were negative by NAT and confirmatory testing (INNO-LIA test). The possible explanations for “False Reactive” results via Serological false positive reactivity might be due to either cross-reactivity or contaminants in coating antigens, or to levels of DNA/RNA concentrations in specimens tested that were below detectable limits by molecular test [6,19,20]. Some patients with discrepant serology vs. NAT results might be “biological false positives” who have cleared their infections while others are “analytic” false positives that, in the absence of past infection, have positive serology due to cross-reactivity or other factors that reduce the specificity of the assay [21,22]. This suggests high specificity for NAT excluding HCV-viremia in spite of the presence of non-specific antibodies detected by serological testing [21,22].

‘False Positive’ serology for anti HCV can be seen in patients who have cleared the virus after an acute infection or by therapy and as such may be positive on serology which could indicate past infection [23,24]. Patients with autoimmune hepatitis and other hyperglobulinemic states may also give false positive results [21].

The remaining discrepant cases were positive for anti-HIV via serology but nonreactive by both NAT and confirmatory serological testing (particle agglutination test). False-positive HIV serology screens are commonly caused by recent influenza vaccination and incidental viral infections [25,26]. In addition false positive anti-HIV antibody has been reported in other conditions such as autoimmune disease, blood transfusion, multiple pregnancies, renal failure, hemodialysis, cystic fibrosis, liver diseases, parenteral substance abuse, early HIV infection, other retroviral infection(s), or vaccinations against hepatitis B and rabies [27].

The only concordant results for NAT and Serology were reactive results for HBV DNA and HBsAg, respectively; results that were not considered window cases.

The NAT was also compared with serological screening for sensitivity and specificity which initially measured 17.3% and 99.6%, respectively, yielding a lower sensitivity for NAT as compared to serology. However, nineteen samples that were initially serologically reactive but NAT non-reactive were also found nonreactive on confirmatory testing. Therefore, the sensitivity of NAT increased considerably (to 100%) after the final serological confirmatory test as compared to the serology screening test. On the other hand, NAT specificity was reduced from 99.60% to 79.20% after the initial five yield cases were unconfirmed by both discriminatory and confirmatory tests.

As a part of the guidelines for any reactive but indeterminate or equivocal result, whole blood units collected from the donor must be quarantined or discarded in order to avoid infusion of a low-level viraemic unit originally detected as ‘reactive’ on initial screening but missed in repeated assays [15]. The major issues requiring solutions here involve indeterminate and equivocal results as they may result in the waste of precious blood and blood products and also require additional measures when counseling donors [28].

5. Conclusion

This study demonstrates that NAT is helpful in the detection of false positive cases, as these samples were confirmed as nonreactive by repeated serological, confirmatory and NAT assays, which serological reactivity also led to lower sensitivity for NAT when compared to serology. However, yield cases were not confirmed as the study was limited by its small sample size, random sampling, and difficulty in donor follow-up.

The five potential yield cases should not be overlooked however. Therefore, we recommend that both tests should run concomitantly in our blood bank. Furthermore, we recommend that a larger and more detailed study may help detect the yield of occult viral infections as demonstrated by other studies across the globe.

Although we met requirements that made this study a success, there were indeed limitations encountered. These included:

(a) Limited budget and small number of cases were the main obstacles. Such limitations may not detect a wider range of infectious donors so that reported findings may not accurately represent the entire population of Kelantan and certainly not Malaysia.

(b) As the majority of samples were taken from ‘regular’ donors to the blood bank, we would need to sample different groups of donors from different areas and age groups in order to increase the study’s significance.

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References