Original Article

Nucleic acid testing: Is it the only answer for safe Blood in India?

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Abstract:

Background: With the implementation of NAT in countries around the world, there is a growing pressure on the transfusion services in India to adopt NAT testing. India has about 2545 licensed Blood Centres. The Transfusion Services in India are fragmented, poorly regulated and the quality standards are poorly implemented. Blood Centres are still dependent on replacement/family donors and in most places laboratory testing for Transfusion transmitted infections is not quality assured, laboratory equipment are not calibrated and maintained, and validation of results is not carried out. Against the current scenario introducing NAT for screening of blood donors in India would pose a challenge. **Aim:** To study the prudence of universal NAT testing in India. **Materials and Methods:** A retrospective study of 5 years from 2008-2012 was undertaken to study the true reactivity of donors using WHO strategy II and III and therefore the true seroprevalence of TTI infections in the donor populations. **Results:** The true reactivity of the donors was much less as compared to the initially reactive donors due to the use of a well designed testing algorithm. In addition having a total voluntary blood collection along with good pre-donation counseling program also reduces the transmission of infections. **Conclusions:** What India essentially needs to do is religiously implement the strategies outlined in the WHO Aide-memoire. The blood should be collected only from voluntary non remunerative and repeat donors , there should be stringent donor selection with pre-donation counseling instituted. Strict implementation of quality management system, development of well defined testing startegies and strong haemovigilance system could take us a step in the right direction.

Key words:

NAT yield, window period, haemovigilance, algorithms

Introduction

With the implementation of nucleic acid testing (NAT) for screening of blood in blood centers of countries around the world such as the USA, Canada, Australia, New Zealand, South Africa, and some countries in Europe and Asia there is growing pressure on the transfusion services in India to adopt NAT.

A number of private hospitals in the urban area have already implemented NAT testing while the some of the trust and the government blood centers are still either carrying out enzyme-linked immunosorbent assay (ELISA) or rapid test.

We, therefore, felt the need to assess the prudence of implementing NAT testing in India vis a vis the current status of transfusion services in India.

India has about 2545 licensed blood centers[1] which include both the private and the government blood centers. Transfusion services in India are fragmented, poorly regulated and the quality standards are also poorly implemented and varying from center to center. Blood centers are still dependent on replacement/family donors who even now contribute 50% of the total blood collection of 9.3 million units of the blood collected in the country.[2] The National AIDS Control Organisation (NACO) report, 2012, indicates the figures for the voluntary donations to be in the range of 84.3%[3] but this could also be due to the fact that some of the blood centers consider relative donors as voluntary donors. Although, paid donors/professional donors have been banned by the Supreme Court ruling of 1st January 1998, these donors still continue to donate blood in the guise of relative donors, thereby, further compromising blood safety.

Donor awareness regarding transfusion transmitted infections (TTI) is poor and behavioral screening of potential donors is also not routinely conducted and, therefore, individuals with high-risk behavior still continue to donate. In addition, mega blood donation

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drives which are often held to celebrate special occasions, further add to our woes, as quality is often compromised at these outdoor blood donation drives.

The testing algorithms for TTI s are also variable. In the urban areas, screening for TTI is carried out by the ELISA technique using assays of variable sensitivity and specificity while the remote rural areas are still left with a rapid test of questionable sensitivity. Laboratory testing for TTI's is not quality assured, laboratory equipment is not calibrated and maintained, and the validation of results is not carried out. Screening of blood is carried out with third generation antibody based ELISA, which do not cut down the window period to as great extent as the fourth generation antigen-antibody tests.

To compound the problem, the technologists are inadequately trained, and the blood center in charges are also usually not qualified/trained in the field. They are often reluctant partners in the service, who could be transferred at any point of time and therefore, they lack the commitment and motivation to work in the field.

Availability of kits and reagents is also dependent on a number of factors. Procuring is done centrally by the government, and then the kits are transferred to various centers. At times, the procurement is irregular, and the cold chain maintenance is also not up to the desired standards.

Finally, the lack of clinical awareness on the rational use of blood leads to unwarranted single unit transfusions, thereby unnecessarily exposing the patients to TTIs.

Materials and Methods

The Indian Red Cross Society, Bombay City Branch, blood center, is a standalone blood center based on 100% voluntary donor program and replacement donors are not accepted.

Besides the medical examination predonation counseling of the blood donors is carried out, TTI awareness leaflets are distributed to the blood donors prior to the holding of blood donation camps. The medico-social worker visits the venue and holds discussions with the prospective blood donors and motivates them to donate blood regularly, and he also educates them on issues of self-deferral and confidential self-exclusion. Information and educative materials are displayed at the venue prior to the blood donation drive.

Screening of blood is carried out with the assays provided by NACO. All the initial reactive units are discarded as per the NACO guidelines but for the sake of post donation counseling the results are confirmed using an algorithm designed by us based on WHO strategy III. All the initially reactive units are tested in duplicate on the same assay and then the units which are reactive on the same assay are tested on two supplemental assays, which are more specific as compared to the initial screening assay.

Based on this methodology a retrospective study of the last 5 years was undertaken at the Indian Red Cross Society, Bombay City Branch, Blood Center to assess the true reactivity of the donors using the WHO strategy II and III; and therefore, the true seroprevalence of TTI infections in the donor population.

Observations

During the course of our study we have observed that the number of units which were confirmed as truly reactive are much lesser than the initially reactive units, this clearly indicates that the actual seroprevalence of TTI in India is much lower than that indicated in the NACO figures[4] which is 0.2% for HIV, 1.4% for hepatitis B virus (HBV), and 0.4% for hepatitis C virus (HCV).

As seen from Table 1, it will be observed that the seroprevalence in blood donors at IRCS for HIV is 0.08%, HCV is 0.09%, and HBV is 0.70%, which is much lower than NACO reported prevalence. (The initial reactive figures at our center are high as we used the value of 20% of the cut off instead of 10% and all the tests have been carried on third generation assays).

This clearly indicates that having a well-defined testing algorithm will help in getting the true seroprevalence of TTIs in the blood donors of our country. In addition having total voluntary blood

Table 1: Seroprevalence at IRCS, blood center Mumbai

Year	Total collection	HIV		HCV		HBV	
		Initial reactive	Confirmed reactive	Initial reactive	Confirmed reactive	Initial reactive	Confirmed reactive
2008	12,275	0.35	0.15	0.45	0.10	1.16	0.77
2009	6567	0.15	0.07	0.44	0.14	0.76	0.61
2010	12,453	0.47	0.10	0.50	0.10	1.08	0.67
2011	11,775	0.43	0.05	0.50	0.03	1.23	0.82
2012	12,950	0.80	0.04	1.03	0.08	0.98	0.66
	Average %	0.44	0.08	0.58	0.09	1.04	0.70

IRCS: Indian Red Cross Society, HIV: Human immunodeficiency virus, HCV: Hepatitis C virus, HBV: Hepatitis B virus

Table 2: Window period for the 3rd/4th generation/ID NAT versus the cost

Infectious marker	Window period			Cost		
	3rd generation test	4th generation test	ID NAT	4th generation test	ID NAT	MP NAT
HIV	21 days	14 days	6 days	Rs. 33/-		
HBV	37 days	24 days	15 days	Rs. 28/-		
HCV	59 days	10 days	4 days	Rs. 140/-	Rs. 1450/-	Rs. 1000/-

ID NAT: Individual donor nucleic acid testing, HIV: Human immunodeficiency virus, HCV: Hepatitis C virus, HBV: Hepatitis B virus, MP: Minipool

collection along with good predonation counseling program also reduces the transmission of infections.

From Table 2, it will be noticed that there is only marginal reduction of the window period of 8 days, 9 days, and 6 days for HIV, HBV, and HCV, as against the amount of money that will be spent on testing one unit of blood.

In India, studies conducted at Apollo Hospital,[5] AIIMS, Max Health Care in Delhi and Hinduja Hospital in Mumbai as seen from Table 3 reveal that the NAT yield for HIV, hepatitis B, and hepatitis C is very low.

Discussion

A comprehensive strategy for blood safety was released by WHO in 2002 to guide the developing countries for the implementation of their National Blood Programme.[6] This strategy included the following four key elements to implement the blood safety program in a country:

1. The establishment of a nationally coordinated blood transfusion service.
2. Collection of blood from voluntary nonremunerated low behavioral risk donors.
3. Implementation of universal blood screening for TTIs.
4. Reduction in unnecessary transfusions.

Basavaraju *et al.*[7] observed that in1994 the prevalence of HIV amongst Kenyan blood donors was 6.4 % and incidence of the transfusion-transmitted HIV in the population was also 2% which was brought down to 1.2% and 0 %, respectively, in 2009, only because of strict implementation of the WHO strategy. They further carried out a study to assess the benefits of implementation of NAT vis-à-vis fourth generation ELISA testing and concluded that there was no additional safety benefit of NAT. This clearly indicates that in developing countries where the resources are limited, simple implementation of WHO strategy could help to reduce the incidence of transmission of TTIs.

In a study conducted by Makroo *et al.*[5] it was reported that the NAT yield was higher at hospital-based centers, as they had a larger proportion of replacement donors as compared to stand alone centers who had a higher proportion of voluntary donors. This further affirms that having 100% voluntary blood donation program with good predonation screening and counseling and having good blood donor relation programme will definitely help to reduce the incidence of transmission of TTI.

Implementation of NAT is argued on the grounds that NAT reduces the window period detection, thereby making blood much safer. As seen in Table 2, it will be observed that there is only marginal reduction of the window period of 8 days, 9 days, and 6 days for HIV, HBV, and HCV as against the amount of money that would be spent on testing one unit of blood.

Table 3: NAT yield of the different blood centers of India

TTI	Apollo hospital (%)	AIIMS (%)	Max (%)	Hinduja (%)
HIV	0.016	0.09	0.019	0
HBV	0.04	1.05	0.002	0.005
HCV	0.008	0.25	0.002	0
Total	0.065	1.49	0.023	0.005

HIV: Human immunodeficiency virus, HCV: Hepatitis C virus, HBV: Hepatitis B virus, NAT: Nucleic acid testing, AIIMS: All India Institute of Medical Sciences

Thus, the cost for 1 unit of blood will be: Rs.1450 for NAT + Rs.201 for ELISA = Rs.1651.

It is a known fact that NAT simply cannot be used as a screening test, as at times the viral load may go down to undetectable levels by NAT but the antibody response can still be present and detected by ELISA. Therefore, NAT has to be used as add on the test, which would then further entail an additional cost.

The cost of 9.2 million units that is the number of units collected in the country would be 90,000,00 units × Rs 1651 = Rs.1485 crore.

Over and above this, the cost for infrastructure, additional staff, and their training would be required.

In India, studies conducted at Apollo Hospital,[5] AIIMS, Max Health Care in Delhi and Hinduja Hospital in Mumbai as seen in Table 3 reveal that the NAT yield for HIV, hepatitis B and hepatitis C is very low. The additional yield refers to the infection that is not detected by ELISA, but detected by NAT. It will be noticed that the sensitivity and specificity of NAT are both high, but the prevalence rate of the TTI's is low in India. Therefore, the effective yield is also low.

The only problem is that of occult hepatitis, non-seroconverting or delayed seroconverting individuals. Hence, NAT testing is recommended for hepatitis B on the grounds that ELISA does not pick up occult hepatitis. We need to understand that India is a country with a moderate prevalence of hepatitis B, and therefore there is a real need to have an updated data on the number of such donations.

Thoai Duong Ly[8] observed that the new generation of hepatitis B surface antigen (HBsAg) assays use mixture of monoclonal antibodies in order to recognize the known "S" gene mutant and ideally they should be sensitive enough to detect the smallest amount of HBsAg present in the low-level carriers.

Occasionally false negative results with NAT have also been observed, Delwart *et al.*[9] have reported transmission of HIV-1 from NAT negative donations with low viral load. Barbara Foglieni *et al.*[10] have commented that the increased heterogeneity of the HIV virus could have led to the false negative results thereby affecting the safety of blood supply as well as the diagnosis and patient management. This demonstrates that despite the employment of most sensitive type of assays, zero risk is unattainable as no single test is foolproof.

Another area of concern is the false positivity with NAT. The false positive rate in a study carried out by Stramer *et al.*[11] (i.e. unconfirmed NAT reactive donations) was 1 in 15,800 units. In India too, the study of Makroo *et al.*[5] revealed that 27 samples out of 12,224 were Ultrio reactive but negative on discrimination test. During the study, they could repeat only five of these samples again, and these were found to be negative on Ultrio again. They were, however, unable to repeat the rest 22 samples as the samples were inadequate.

In a study published by Stramer[12] that tested samples for HIV and HCV NAT, there were 193 donations that were reactive for NAT, of these 92.2% were false positive. Kleinman *et al.*[13] have reported out of the 23 HBV DNA positive, HBsAg, and antibodies to hepatitis B core antigen negative, only two were confirmed positive resulting in 91.3% false positive rate.

The reason for these false positives has been best explained by Pisani *et al.*[14] in their study, which states that false positive results with external proficiency samples of NAT are often attributed to the cross-contamination. In a checkerboard distribution of virus positive and virus negative samples cross-contamination rates of 4.2 or 8.3% have been reported.[15]

Further, there is a constant debate on which one is better - minipool (MP) NAT or individual donor nucleic acid testing (ID NAT).

A recent study suggests that the dilution factor involved in MP testing can certainly not detect infectious donations especially when the viral load is low[16] and hence ID NAT may be required to identify rare window phase donations. At the same time, another recent case report[17] have revealed that even ID NAT may not be able to detect all the infectious units as only a small volume of plasma is tested in comparison to the volume of the blood that is being transfused.

In a study authors[18] have expressed their concern over the fact that ID NAT is capable of detecting exceedingly rare cases of viral transmission.

A NAT study group was established in USA whose key objective was to develop quantitative data on the dynamics of viremia during the early phase of HIV, HCV, HBV infections with particular focus on the issue of the relative capacity of MP and ID NAT to infectious window period. The study involved analysing the performance of quantitative and donor screening NAT assays on a serial donation from several hundred source plasma donors who seroconverted or were detected during the Viremia phase by NAT screening in that model.[19] Apart from this, studies using animal models and recipient look back strategies to determine the infectivity of early window phase donation[16,19,20] have shown that the incremental window period closure and yield of ID over MP NAT for HIV and HCV will be very small and will come at a very high cost.

To study the risk of transmitting HCV/HBV/HIV by blood transfusion in Victoria,[21] the incident rate was calculated as number of incident cases (i.e. the number of seroconverters) divided by the sum of interdonational intervals in person years as described by Busch *et al.*[22] In India too, we need to find out our true seroprevalence and the incidence of infections transmitted through transfusions using the above-mentioned formula.

India would face many challenges for implementation of NAT. Due to poor blood collection at some centers it would not be feasible to carry out NAT testing at all the centers. Hence, regional/zonal blood testing centers would need to be identified. Implementation of NAT would require building of specially designed and equipped laboratories which would mean investment in the infrastructure, equipment and manpower and therein resources required for adopting the same.

Transporting samples from the remote areas to these centers in time will certainly delay the utilization of blood and components, especially platelets which are usually required in a dire emergency. The condition of the samples when they reach due to poor transport infrastructure is also a real area of concern. In the study conducted by Makroo *et al.*[5] it was noticed that out of 12,224 samples, 570 samples could not processed as 350 samples had inadequate blood volume, 176 - had no barcode/were mislabeled, 22 samples were hemolyzed and 22 had shown indeterminate results.

To get the best results, implementation of quality management system and good manufacturing practices are of paramount importance. If stringent quality measures and standards of cleanliness are not observed, then the number of false positives could certainly go high thereby further contributing, not only to blood shortage but also adding to the cost.

Proficiency testing for NAT is not available in India and one needs to depend on expensive panels from abroad therefore quality assurance/quality control is challenged.

Additionally, the technologist will need to be trained in molecular biology techniques, handling of complex new data management systems for sample pooling, resolution, and supplemental testing of reactive pools.

We also need to understand that we do not have any study or data that reveals window period cases that are transmitted undetectable both by NAT and ELISA. One also has to keep in mind that emerging infections are a challenge, and therefore, in spite of NAT testing the window phase cannot be shut, and zero risk supply cannot be achieved.

Conclusion

In the past two decades, there has been a tremendous improvement in the infectious disease screening of blood donations which has resulted in considerable safe blood supply. However, we have still not been able to achieve zero risk blood supply. In our desire to chase this elusive goal, we need to incorporate more scientific data, find out the true incidence rates and seroprevalence rates of infections in the population as well as in the patients who have received transfusions and then carry out cost-benefit analyses before we take any decision on implementation of NAT.

What India essentially needs is, to religiously implement the strategies outlined in the WHO Aide-memoire. We need to focus on the logo "safe donor safe blood." The blood should be collected only from voluntary, nonremunerative and repeat donors; stringent donor selection with predonation counseling should be instituted. Strict implementation of quality management system, training of the staff, and a good hemovigilance system could take us a step in the right direction.

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Conflicts of interest

There are no conflicts of interest.

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