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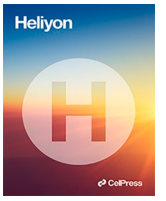

Research article

Efficacy of combined HBsAg, anti-HBc and anti-HBs screening in minimizing transfusion transmission risk of hepatitis B infection in low resource setting

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ABSTRACT

Background: Hepatitis B Virus (HBV), and occult Hepatitis B in particular, is a major concern in the transfusion scenario, especially in endemic countries. This study attempted to estimate the prevalence of occult Hepatitis B infection (OBI) among voluntary blood donors in Maharashtra and to evaluate the role of combined screening strategy with implications in minimizing the current transfusion risks of seropositive OBI.

Methods: Donor samples were collected from 80 eligible blood banks from various districts of Maharashtra between 2014 and 2017. ELISA based screening of HBsAg, anti-HBc (total and IgM), anti-HBs titres. Real-time quantitative PCR for Hepatitis B Virus DNA (HBV DNA) were performed for all HBsAg and or anti-HBc positive samples.

Results: Out of 2398 samples tested, 20 (0.83%) samples were positive for HBsAg, whereas 547 (22.81%) were positive for anti-HBc. Out of 547 samples, 16 (2.92%) were positive for HBV DNA with median level at 247.89 IU/mL (IQR: 126.05–666.67 IU/mL). Anti-HBs levels were positive in 35.83% of OBI cases. ROC curve analysis showed that combined HBsAg, anti-HBc and anti-HBs (>50 mIU/mL) screening can more efficiently detect HBV infection in blood donors than HBsAg alone.

Conclusions: A combined HBsAg, anti-HBc and anti-HBs screening for donor samples could be an alternative achievable strategy to minimize the HBV transmission as well as financial burden. In resource limited setup, the proposed combined strategy could be helpful in minimizing the risk of OBI transmission.

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1. Introduction

According to the World Health Organization's (WHO) estimates, 118.4 million blood units are collected globally every year [1]. In India, 14.6 million units of blood are needed annually to fulfill the demand for blood across the healthcare systems. However, the National AIDS Control Organization (NACO) reported an annual collection of 11.1 million units in 2017, which may not be enough to fulfill the demand due to inequitable distribution across the country [2]. WHO guidelines mandate the screening of donated blood units for transfusion-transmitted infections (TTIs) such as HIV, HBV, HCV, and Syphilis to ensure safe blood transfusion [1]. The Drug and Cosmetics Act of India also makes it mandatory to screen for TTIs before transfusion [3]. Hepatitis B is a major cause for concern, with about 1.5 million new infections each year globally, and the potential to cause liver related complications, including hepatocellular carcinoma. Currently, about 296 million people have been infected with the virus globally, with an estimated 8,20,000 deaths due to HBV occurring annually [4–6]. The prevalence of HBV in India ranges from 1.4 to 2.7% [7] [INASL Statement, 2018] and an estimated 1,15,000 Indians succumb to HBV-related complications every year [8].

While the implementation of screening strategies using sensitive ELISA and nucleic acid testing (NAT) assays for transfusion transmitted infections(TTI) screening has significantly reduced the per-unit infection risk for HIV, HBV and HCV in the past 30 years, the risk of transmission of occult HBV infection is still a matter of debate [9]. The estimated risk of transmission of HBV due to occult HBV infection has been around 3% in countries with low endemicity [10,11]. Recently, the minimal HBV infectious dose of HBV DNA was reduced from 20 IU/mL to 3 IU/mL, which is below the limit of detection of currently available NAT assays (3.4 IU/mL) [12]. Donor samples with HBV can be missed in case of a window period of infection, recovery stage with minimal HBV replication, and the presence of escape variants [13]. The international workshop report on occult HBV in 2018 suggests that the estimated transfusion transmission risk of OBI may be underrated; thus, the actual incidence of OBI-related transmission could be higher. This highlights the importance of OBI screening to ensure transfusion safety. Based on serological profile, two types of OBI have been defined, namely, seropositive(HBsAg negative, anti-HBc and or anti-HBs positive) and seronegative(HBsAg negative, anti-HBc and anti-HBs negative) [14].

In a country where most of the blood bank units operate in a resource-limited setup, NAT testing is a costly and non-viable [15]. Though pooled NAT can be relatively cost effective over individual NAT testing, but it reduces the sensitivity of NAT testing and thus fails to identify OBI [16]. Several countries use different combined HBsAg, anti-HBc, anti-HBs and or NAT testing for improved transfusion safety. However, no such study has been reported from India.

The present study was designed to understand the prevalence of OBI among voluntary blood donors in Maharashtra and to evaluate the combined serological screening strategy employing HBsAg, anti-HBc and anti-HBs levels for identifying OBI and improving transfusion safety. The objective was to provide insights into the current scenario concerning OBI in the country and provide information to improve the current blood screening strategies in the country.

2. Materials and methods

2.1. Sample collection

This study was conducted at the Department of Medical Biotechnology, MGM University of Health Sciences, Navi Mumbai from 2014 to 2017. The study was approved by the Institutional Ethics Committee, MGM Institute of Health Sciences (MGM/HIS/RS/2014/112 dated 11.08.2014) and informed consent was taken from the participants before enrollment. The serological and molecular testing of the samples was conducted at the Transfusion Transmitted Diseases Department, ICMR-National Institute of Immunohaematology. Around 2 mL of 200 serum samples used for TTI testing were requested from 80 eligible blood banks from various districts of Maharashtra after prior permission from Maharashtra State AIDS Control Society (MSACS) and State Blood Transfusion Council, Maharashtra. Around 2 mL of plasma samples, negative for HIV/HCV were collected and transported under cold chain conditions to ICMR-National Institute of Immunohaematology, Mumbai for serological and molecular testing. The samples were divided into 2 aliquots of 500 μL and 1.5 mL each and stored at −20 °C and −80 °C till further testing.

2.2. Operational definitions

HBsAg is the marker used for screening Hepatitis B. Samples positive for HBsAg were considered as overt HBV infection. HBcAb or anti-HBc indicates antibodies to the core antigen of HBV. HBV DNA indicates the nucleic acid of HBV. Samples positive for anti-HBc, but negative for HBsAg along with positivity for HBV DNA were considered occult HBV infection. Anti-HBs or HBsAb indicates antibodies to the surface antigen of the HBV. In chronic HBV cases, the appearance of anti-HBs along with the absence of HBV DNA indicates seroconversion and clearance of HBV. The presence of only anti-HBs without any other markers could indicate protection due to vaccination.

2.2.1. Serology and molecular testing

The samples were tested for detection of HBsAg (SD HBsAg ELISA 3.0, Standard Diagnostics), and anti-HBc (MBS HBcAb one step ELISA, MBS Italy) using ELISA. All samples testing positive for HBsAg/anti-HBc were repeat tested using the same ELISA kits. Additionally, samples positive for anti-HBc were also tested for anti-HBc IgM (HBcIgM one step ELISA, MBS Italy) and anti-HBs (MBS HBsAb ELISA kit, MBS Italy and HBsAb Quantitative ELISA, Diapro Italy) using ELISA. HBsAg/anti-HBc positive samples were quantitatively measured for HBV DNA using commercially available Real-time quantitative PCR (HBV DNA Quantitative PCR, MyLab

Life Solutions). The analytical sensitivity of the HBsAg, and HBcAb, ELISA kits was 0.2 IU/mL and 1 PEI U/mL. The standards used for HBsAb quantitation ranged from 0 to 250 WHO mIU/mL. The minimum detection limit of Real-time quantitative PCR was 10 IU/mL.

2.3. Statistical analysis

The prevalence of HBsAg and anti-HBc was computed with respect to total number of donor samples collected and expressed as a percentage. The data was compiled and analyzed using Microsoft Excel and SPSS version 20. Receiver operating characteristic (ROC) curve was plotted to compare the efficacy of combined screening and HBsAg alone taking HBV DNA estimation as gold standard.

3. Results

A total of 2398 samples were collected from 14 districts of Maharashtra. Out of these, 20(0.83%) samples were positive for HBsAg, whereas 547 (22.81%) samples were positive for anti-HBc only, as shown in Fig. 1. As seen in Table 1, the prevalence of HBsAg and anti-HBc varied across districts, with the highest prevalence of HBsAg observed in Buldana 6(7.06%), followed by Osmanabad 3(1.9%) and Nanded 3(1.6%). In contrast, samples collected from Nagpur, Kolhapur, Sangli, Thane, Jalgaon, Aurangabad did not show any HBsAg positive samples. The highest prevalence of anti-HBc was observed in Jalgaon 88(47.31%) followed by Pune 53(40.46%), Osmanabad 63(39.87%) and Aurangabad 33(39.76%). Anti-HBc IgM was not detected in any of the samples

Out of the 547 samples positive for only anti-HBc, HBV DNA was detected in 16 (2.92%) samples. The median viral load detected in the samples was 247.89 IU/mL (IQR: 126.05–666.67 IU/mL). Only 1 of the out the 16 samples had HBs Ab titers above 50 mIU/mL. Two samples borderline positive for anti-HBc and negative for the other serological markers showed presence of HBV DNA.

The anti-HBs antibodies were tested in the 547 anti-HBc only positive samples. Raigad had the highest anti-HBs positivity among anti-HBc positive cases. The distribution of anti-HBs was varied among the districts, with percent positivity ranging from 18.18% to 66.67%. As shown in Fig. 2, out of the 196 samples positive for anti-HBs, 117 (59.69%) had titers between 10 and 50 mIU/mL while only 79 (40.3%) had titers above 50 mIU/mL.

As shown in Table 2, the combined screening of HBsAg, anti-HBc and anti-HBs titres >50mIU/mL provided a higher sensitivity as compared to HBsAg alone. The area under the curve of all the models was determined using the ROC curve and it was found that anti-HBc and anti-HBs titres >50 and > 100mIU/mL had an AUC of 0.873 and 0.880 respectively, as seen in Fig. 3. The sensitivity, specificity, negative predictive value, and the standard error for each model is provided in Table 2.

4. Discussion

HBV remains the most frequent transfusion transmitted infection. Donated blood negative for HBsAg have the potential for transmitting infection at HBV DNA levels as low as 3.4 IU/mL [12,17]. NAT testing is difficult to conduct in resource-limited settings, due to high cost of implementation and the level of expertise required to perform the testing. Hence, the present study tried to evaluate the prevalence of OBI among voluntary blood donors and the potential utility of anti-HBc and anti-HBs testing in screening OBI cases, especially in resource-limited scenarios.

In the present study, 0.83% of the samples were positive for HBsAg which is similar to other Indian studies [18,19]. However, the prevalence of anti-HBc (22.81%) was higher than most of the other studies across the country, where prevalence is reported between 0 and 15% [20–24]. However, very few studies have been documented from Western India.

The varied prevalence of anti-HBc among different districts in Maharashtra suggests the presence of pockets of HBV in some areas. Factors like population, ethnicity, migrants, customs, living standards, promiscuity, and prevalence of sexually transmitted infections

Table 1
Prevalence of different markers in different districts from Maharashtra.

District	No. of samples	HBsAg positive	Anti-HBc total positives *n* (%)	HBV DNA positive (*n* = 547) *n* (%)	Anti-HBs positive n (%)	No. of Anti-HBs positive cases with titers
		n (%)				>50 mIU/mL *n* (%)
Raigad	382	4 (1.05)	42 (10.99)	0 (0.00)	28 (66.67)	19 (67.86)
Nagpur	80	0 (0.00)	10 (12.50)	0 (0.00)	2 (20.00)	2 (100.00)
Nashik	196	1 (0.51)	34 (17.35)	0 (0.00)	7 (20.58)	6 (85.71)
Kolhapur	200	0 (0.00)	18 (9.00)	0 (0.00)	5 (27.78)	3 (60.00)
Sindhudurg	200	1 (0.50)	18 (9.00)	0 (0.00)	7 (38.88)	3 (42.86)
Osmanabad	158	3 (1.90)	63 (39.87)	7 (4.43)	30 (47.61)	16 (53.33)
Sangli	175	0 (0.00)	67 (38.29)	1 (0.57)	16 (23.88)	6 (37.50)
Nanded	188	3 (1.60)	33 (17.55)	0 (0.00)	20 (60.61)	11 (55.00)
Thane	173	0 (0.00)	22 (12.72)	1 (0.00)	7 (31.82)	6 (85.71)
Dhule	161	1 (0.62)	49 (30.43)	0 (0.00)	21 (42.86)	13 (61.90)
Jalgaon	186	0 (0.00)	88 (47.31)	4 (2.15)	16 (18.19)	11 (68.75)
Pune	131	1 (0.76)	53 (40.46)	2 (1.52)	15 (28.30)	10 (66.67)
Aurangabad	83	0 (0.00)	33 (39.76)	1 (1.20)	14 (42.42)	8 (57.14)
Buldana	85	6 (7.06)	17 (20.00)	0 (0.00)	8 (47.05)	3 (37.50)
Total	**2398**	**20 (0.83)**	**547 (22.81)**	**16 (2.92)**	**196 (35.83)**	**117 (59.7)**

Table 2
Sensitivity and specificity, Area Under the Curve, Standard Error and discard rate for the various screening strategies with respect to HBV DNA detection as the gold standard.

Screening Test	Sensitivity	Specificity	NPV	Std. Error	Rate of discard per 1000 units
HBsAg	52.63%	100.00%	99.24	0.052	8 (0.8%)
HBsAg + anti-HBc	94.74%	77.50%	99.89	0.023	228 (22.8%)
HBsAg + anti-HBc + anti-HBs>10mIU/mL	80.56%	35.59%	96.43	0.040	146 (14.6%)
HBsAg + anti-HBc + anti-HBs>50mIU/mL	97.22%	21.85%	99.15	0.026	179 (17.93%)
HBsAg + anti-HBc + anti-HBs>100mIU/mL	**100.00%**	**16.76%**	**100%**	**0.022**	**191 (19.1%)**

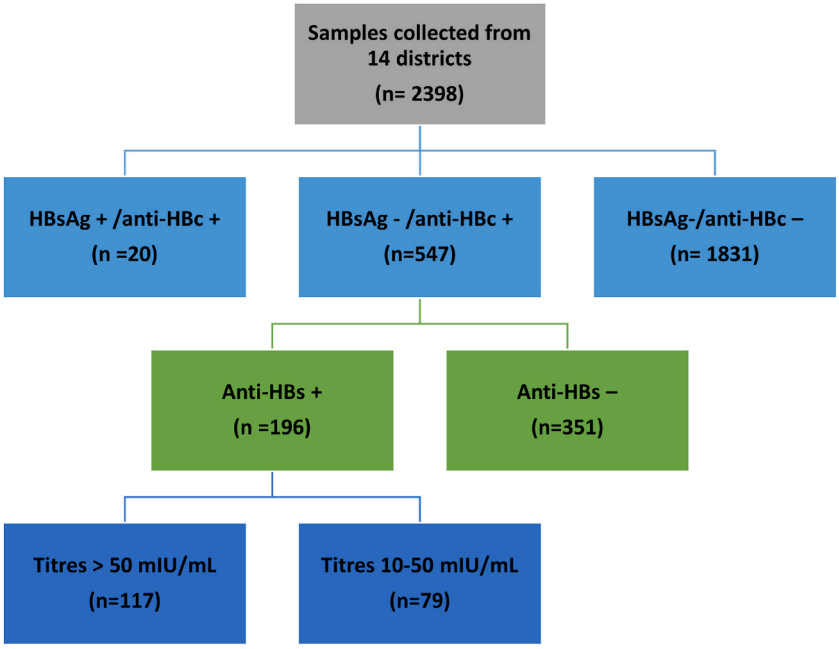

Fig. 1. Screening strategy for serological markers of Hepatitis B.

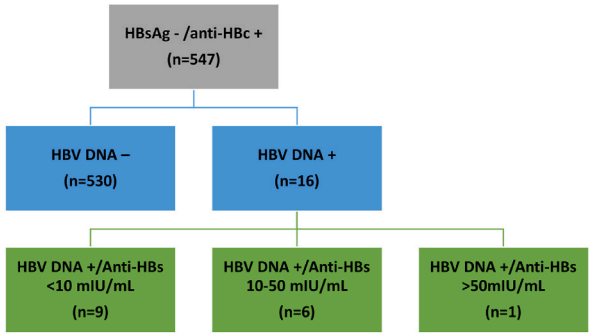

Fig. 2. HBV DNA detection in anti-HBc positive samples.

(STIs) may affect the prevalence of HBV. As observed by Panigrahi et al. the prevalence of OBI along the borders of the state is higher due to higher migration [25]. Maharashtra is also one of the states which witnesses majority of incoming migrant population for employment/marriage [26,27]. On comparing the NACO's epidemiological profile for the districts included in our study, it is evident that districts with out-of-state migration >10% had anti-HBc positivity >20%, indicating migration being one of the driving factors for the higher prevalence [28].

Occult HBV infection was documented in 2.92 % of the anti-HBc positive cases. The OBI prevalence documented across the country varies from 0 to 9% [18,20–22,24,29,30]. However, none of these studies were from Western India. The results in the present study are similar to the prevalence observed in Vellore and West Bengal [24,29,30]. However, one report of Orissa documents a higher

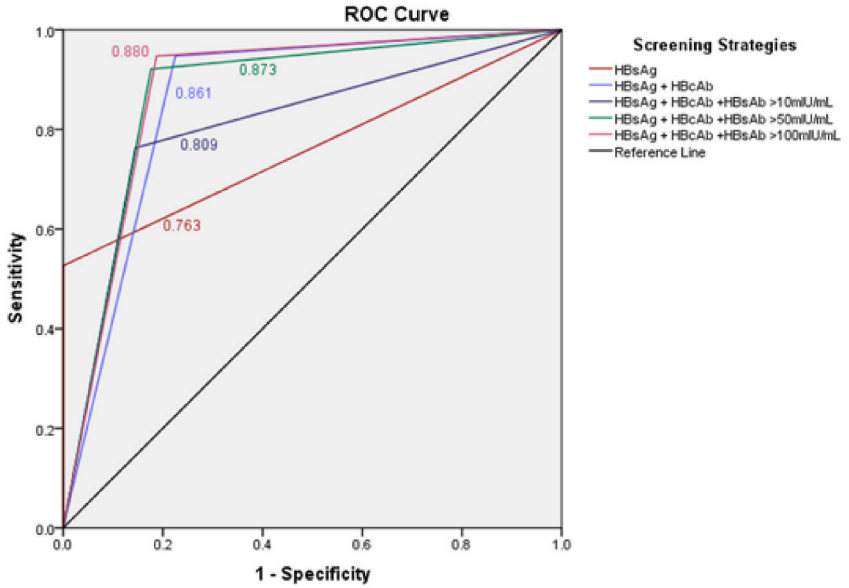

Fig. 3. Receiver Operating Characteristic of different screening strategies to compare the efficacy of combined screening and HBsAg alone with HBV DNA as gold standard.

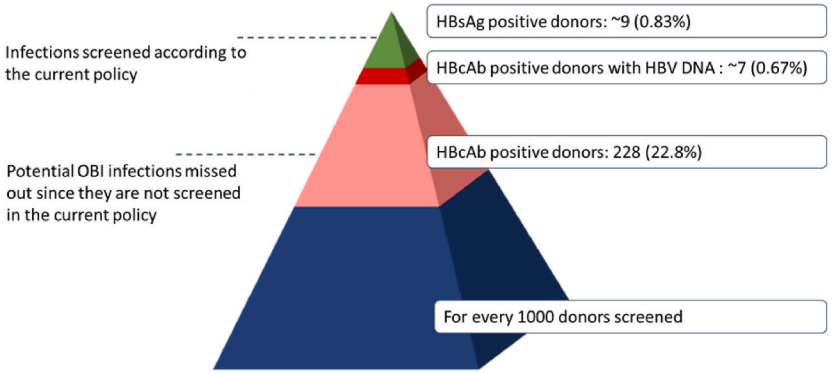

Fig. 4. Estimated number of HBsAg positive and OBI cases per 1000 donors screened.

prevalence of 9%, which is attributed mainly to the migrant population and promiscuous behavior among the individual, increasing the risk of transmission [25].

Additional testing of anti-HBc positive samples with anti-HBs showed that 35.83% of the samples were positive for anti-HBs(titres above 10 mIU/mL), indicating possible recovery from the infection. The finding are in agreement with other studies from India with regional variation of 19%–66% for anti-HBs positivity across the country [18,25]. Out of these seropositive potential OBI with positive anti-HBs, 59.7% of the samples had titres above 50 mIU/mL.

Our findings suggest that only about 1% (i.e., about 9 donors per 1000) of the infections are caught during the serological screening for HBsAg. As shown in Fig. 4, current policy misses about 22.8% of anti-HBc positive donors, 3 % of whom may have a higher risk of transmission due to presence of HBV DNA. A recent study on Swiss blood donors found that some occult HBV infections with potential transmission risk were missed by HBsAg and ID NAT [31]. Introducing anti-HBc testing along with ID-NAT testing can greatly reduce the risk of transfusion-mediated transmission. However, in countries like India where HBV prevalence is high, donor deferral due to anti-HBc positivity alone could severely affect the donor pool [15,32]. So, an estimation of residual risk of transmission of hepatitis B virus is needed to assess the usefulness of anti-HBc in routine screening.

Studies show that having a high anti-HBs titer can significantly minimize the risk of HBV transmission by fivefold [33]. Countries across the world are continually reviewing strategies to screen for OBI, which includes anti-HBc and anti-HBs screening. However, there is lack of consensus on determining the cut-off of anti-HBs preventing HBV transmission. Countries such as France, Slovenia and Japan have set anti-HBs cut-offs of 500 IU/L, 100 U/L, and 200 IU/L respectively for donor deferral in case of anti-HBc positivity [34,35]. In our study, we found that anti-HBc positive donor samples with anti-HBs titer >50 mIU/mL were more likely to be negative for HBV DNA than those with anti-HBs titre <50 mIU/mL (Table 2). The ROC curve analysis, for combined HBsAg, anti-HBc and anti-HBs

at 50 mIU/mL could detect 87% of HBV positive cases as compared to only 76% cases with HBsAg alone. Additionally, this strategy would be able to salvage about 5% of the donated blood deferred due to anti-HBc positivity, thus reducing the discard.

The estimated cost of introducing anti-HBc and anti-HBs in the current screening strategy would be around 25 lakhs INR for 1000 samples. On the contrary, the cost of ID-NAT testing would be around 1.3–1.5 crores INR, which is 5 times more expensive than serological tests. A detailed cost-effectiveness analysis is needed to compare the cost of introducing serology tests with NAT. A previous report by Naidu et al. showed that the introduction of NAT testing will incur an additional cost of Rs. 1450 per unit of blood as compared to ELISA which costs around Rs. 200, excluding the cost of infrastructure, skilled manpower, and other associated expenses [36]. According to the results of our study, we propose a modified combined screening strategy as shown in Fig. 5. Extensive validation and a detailed cost-effectiveness analysis is needed before the introduction of the additional serology tests, however, there is a need to consider such modified algorithms to reduce the risk of OBI transmission.

Our study has some limitations. It was limited by the sample size, unavailability of donor vaccination details, and incomplete coverage of the entire state due to logistic constraints. Larger sample size could have better understanding of the risk of transmission. Also, information regarding total blood collections at the participating centers could have provided further information about residual risk. Detailed information regarding the type of donors as well as in-depth follow-up of donor recipients could have helped in understanding the HBV transmission dynamics. HBV DNA was not measured in ant–HBc negative(potential seronegative OBI) donor samples. Additionally, the limit of detection of HBV DNA for our study was 10 IU/mL, which could have missed potentially infectious donor samples below this limit.

5. Conclusion

Despite the limitations, our study provided important data with respect to prevalence of HBV markers in blood donors in 14 districts of Maharashtra. The results emphasize the need to revise our blood safety policies. To our knowledge, this is the first study from India evaluating the efficacy of combined screening strategy for donor screening. Minipool NAT, though relatively cost saving, is limited by false negativity specially in samples with low but transmissible viremia [37]. ID-NAT, though a better option, is expensive for routine screening and can still miss some low viremic OBI cases. Therefore, our proposed combined strategy can improve the transfusion safety by identification and deferral of seropositive OBI donors. It will slightly increase the current HBV screening cost of HBsAg alone but it will definitely be much cheaper than NAT which is expensive and needs expertise. Hence, this novel, affordable and sensitive screening protocol, that reduces unnecessary wastage due to false positivity while preventing the risk of OBI transmission, is required to improve overall blood safety in a large country like India, especially in resource limited settings.

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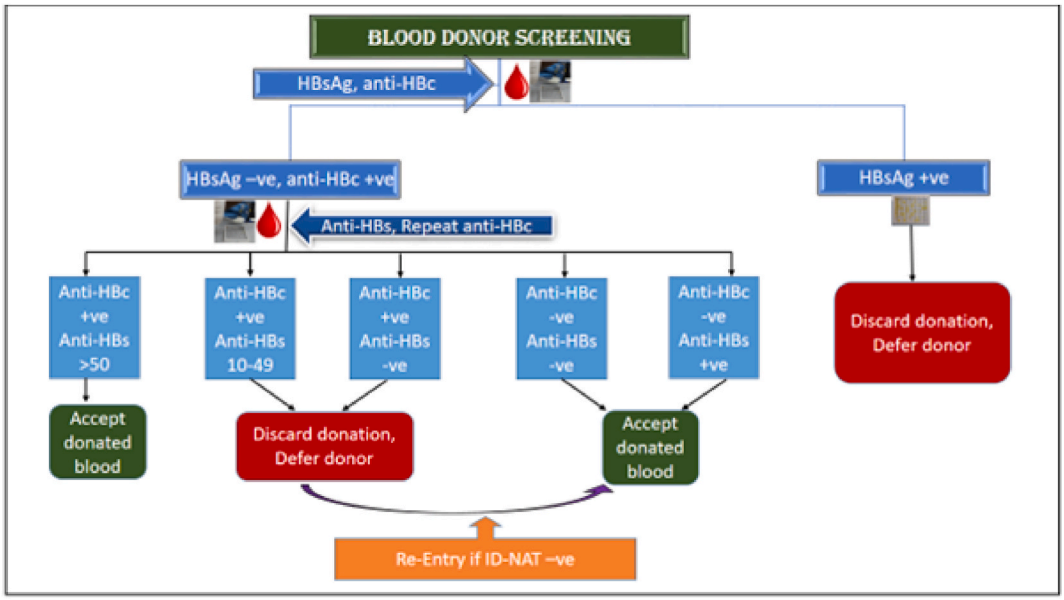

Fig. 5. Proposed OBI screening strategy for in lieu of ID-NAT.

Data availability statement

The data underlying this article will be shared on reasonable request to the corresponding author.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Shreyasi Athalye: Writing – review & editing, Writing – original draft, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Amruta Patil:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Naveen Khargekar:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Project administration, Conceptualization. **Shailesh Shinde:** Writing – review & editing, Supervision, Resources, Methodology, Investigation. **Shreya Chavan:** Software, Resources, Methodology, Investigation, Data curation. **Abhay Dixit:** Writing – review & editing, Software, Resources, Project administration, Data curation. **Aruna Shankarkumar:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Data curation, Conceptualization. **Manisha Madkaikar:** Writing – review & editing, Supervision. **Anindita Banerjee:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Dr Aruna Shankarkumar reports financial support was provided by National Health Mission, Maharashtra. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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