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Original Article

Point of care hemoglobin testing in plateletpheresis donors: Noninvasive versus invasive methods

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ABSTRACT

Background: Predonation hemoglobin (Hb) for plateletpheresis donors is estimated by presently available invasive methods. Venous samples of only those donors whose Hb is more than 12.5 g/dl are screened for complete blood count and transfusion transmissible infections. There is a pressing need to substitute this invasive Hb test with noninvasive one to reduce donor discomfort and avoid further pricking the donor. We therefore went ahead with the aim of comparing a noninvasive Hb estimation method NBM 200 with the invasive method – Hemocue, taking SYSMEX KX-21 as a gold standard.

Methods: 500 voluntary consenting plateletpheresis donors qualifying the laid down criteria for platelet donation were included in the study. Hb readings obtained by the NBM 200 and Hemocue were compared to those obtained from the fully automated hematology analyzer SYSMEX KX-21.

Results: Coefficients of correlation were found to be statistically significant at <0.0001 level of confidence. Results of Friedman's test on the three methods also showed significant difference in means. Bland–Altman plots and mountain plots also confirm the same. NBM 200 was found to be more sensitive, specific, and precise than Hemocue in detecting ineligible donors.

Conclusion: NBM 200 was found to be more sensitive, specific, and precise as compared to Hemocue for predonation screening of Hb in plateletpheresis donors and the prime benefit it offers is that it is 'noninvasive' thereby assisting in stemming the platelet donor pool. The onus lies on the blood transfusion services to make use of appropriately validated gadgets that reduce the donor discomfort.

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Introduction

Plateletpheresis is a procedure by which platelets are harvested from a healthy volunteer donor with the help of a cell separator machine. The product thus obtained is called a Single Donor Platelet unit with a minimum platelet count of 3×10^{11} in 250–300 ml of plasma.¹ This product is unambiguously indicated for thrombocytopenia in immunocompromised and multitransfused patients in order to play down effects of alloimmunization, and since the product is leucodepleted, the adverse effects of donor leucocytes are also avoided.² However, a sizeable effort is required to persuade healthy donors for the harvesting procedure, as they easily get disheartened due to the comprehensive preliminary screening procedures mandated by regulatory authorities (vein status, medical examination, hemoglobin (Hb) estimation, platelet count, screening for transfusion transmitted infections, and blood grouping) and due to the protracted harvesting procedure on the apheresis machine associated with few adverse effects.³

After the initial medical examination, a preliminary screening Hb test is done by the currently available invasive point of care testing (POCT) methods (Hb spectrophotometry). This also gains importance from the fact that anemia is widely prevalent in India and low Hb is the most common cause of temporary deferral of blood donors.⁴ Further screening tests are carried out only if the Hb is above the mandatory level of 12.5 g/dl. There is a burning need to surrogate this initial invasive Hb test with a noninvasive one in order to lessen the donor discomfort as the finger prick is reported as one of the most feared and painful parts of blood donation. Moreover, a few studies have cast doubt over the reliability of the invasive capillary methods.⁵ Thus, donors rejected at this stage would undergo no invasive test at all, and donors selected at this stage would undergo one less invasive test. A recently introduced noninvasive Hb estimation technique based on optical signal measurement after temporary vascular occlusion (occlusion spectroscopy) using a finger sensor probe containing a pneumatic cuff is gaining reputation as the initial noninvasive Hb screening method in blood donors based on numerous studies carried out in the western countries. However, only a few studies have been carried out in India so far and that too only on the whole blood donors.

The aim of the study was to compare a novel noninvasive method for initial screening of Hb, with the conventional invasive method for Hb in screening voluntary plateletpheresis donors.

Material and methods

The sample size calculated was 117 considering the sensitivity of NBM 200 and Hemocue 201+ of 71.7% and 86.8% respectively, prevalence of donor deferral based on low Hb (below 12.5 g/dl) of 18.81%, and an acceptable deviation of 10%.^{6,7} However, after obtaining clearance from the Institutional Ethical Committee, this prospective study was conducted on a larger sample size of 500 consenting donors who came forward for platelet donation at our apheresis center from March 2014 to

August 2015. Informed consent was obtained not only for the study but also for the predonation screening and eventually the plateletpheresis procedure itself.

Noninvasive Hb estimation by NBM 200

The donor Hb was first estimated by the noninvasive Hb estimating method using NBM 200 (OrSense, NesZiona, Israel). This portable instrument operates by means of “Occlusion Spectroscopy” wherein a ring-shaped sensor that is applied over the thumb occludes the local blood flow temporarily and the sensor measures the light transmitted through the finger spectrophotometrically.⁸ The same is projected onto a portable monitor to which the sensor/probe is attached. NBM 200 performs a self-test every time it is started, thereby ensuring quality control of the product, as per the manufacturer's instructions.

Hb estimation by invasive method (Hemocue 201+)

The same donor was then subjected to measurement of Hb by the invasive Hemocue 201+ wherein a drop of blood (capillary blood) obtained after a sterile lancet finger prick was placed onto a cuvette precoated with the reagent buffer solution. Sodium deoxycholate present on the cuvette causes hemolysis of the red cells liberating Hb, which further combines with sodium nitrite to form methemoglobin. Methemoglobin then gets bound to sodium azide forming azide-methemoglobin. The absorbance of light passing through this hemolyzed sample is measured at two wavelengths (565 and 880 nm) to facilitate compensation for turbidity. This yields the Hb concentration of the sample.⁹ The calibration of the equipment was done as per the manufacturer's instructions.

Estimation of venous Hb by automated hematology analyzer (SYSMEX KX-21)

Venous samples of the same prospective platelet donors were then collected using sterile precautions into an ethylene diamine tetraacetic acid vacutainer for Hb measurement using SYSMEX KX-21, an automated hematology analyzer used routinely for in vitro diagnosis. This is a non-cyanide method, which uses sodium lauryl sulfate (SLS) as the hemolyzing agent. The released hemoglobin binds to SLS and forms SLS-Hb, the concentration of which is measured as light absorbance at 555 nm.¹⁰ Calibration of the analyzer was done on a regular basis using EIGHTCHECK-3WP as the quality control material recommended by the manufacturer. This method also served as a reference method for comparing Hb values obtained by previously mentioned two Hb determination methods.

Statistical analysis

The Hb measurement data so obtained was analyzed using SPSS 22. The nature of data was studied using descriptive statistics. A frequency distribution of the Hb measurements was done into three groups, viz. <11 g/dl, 11.1–12.4 g/dl, and >12.5 g/dl. The results obtained by the three methods were subjected to an array of statistics using Friedman's test,

Table 1 – Frequency distribution.

Hb (g/dl)	SYSMEX		Hemocue		NBM 200	
	Frequency	%	Frequency	%	Frequency	%
≤11.00	5	1.0	2	0.4	1	0.2
11.01–12.40	24	4.8	20	4.0	26	5.2
≥12.50	471	94.2	478	95.6	473	94.6
Total	500	100.00	500	100.00	500	100.00

intraclass correlation coefficient, concordance correlation coefficient (CCR), and kappa values. The sensitivity and specificity of NBM 200 and Hemocue were measured using SYSMEX KX-21 as gold standard. Bland–Altman graphs were then plotted wherein the differences between the two techniques were plotted against the average of two techniques. Subsequently, Mountain Plots were created by computing a percentile for each ranked difference between the NBM 200 and Hemocue with the SYSMEX KX-21. In order to acquire the folded plot, necessary transformation was performed for all percentiles above 50 and these percentiles were then plotted against the differences between the two methods.

Results

The mean (SD) of Hb values obtained by the NBM 200, Hemocue, and SYSMEX KX-21 were 14.49 (1.22), 15.00 (1.25), and 14.81 (1.41), respectively. The mean value of Hb obtained by Hemocue 201+ is closer to that of the gold standard SYSMEX KX-21, thereby showing that Hemocue 201+ is more accurate than NBM 200. The SD of Hb values obtained by NBM 200 was less than that obtained by Hemocue 201+, signifying that NBM 200 was more precise than Hemocue 201+. The coefficient of variation of the Hb estimation by Hemocue 201+ was marginally lower (8.36) than NBM 200 (8.39), thereby indicating that Hemocue 201+ is a better technique than NBM

200, although not by much. The frequency distribution of Hb values was done into 3 groups, viz. <11 g/dl, 11.1–12.4 g/dl, and >12.5 g/dl, as shown in Table 1. The number of platelet donors who had their Hb values below the mandatory level of 12.5 g/dl was 27 (NBM 200), 22 (Hemocue) and 29 (SYSMEX KX-21). The sensitivity of NBM 200 [(70.59%) (95% CI 52.52–84.90)] was found to be higher than Hemocue [(50%) (95% CI 32.43–67.57)] in detecting the ineligible donors (ID). The specificity of NBM 200 [(98.93%) (95% CI 97.51–99.65)] was found to be marginally higher than that of Hemocue 201+ [(98.50%) (95% CI 96.93–99.39)]. The positive predictive value (PPV) and negative predictive value (NPV) of NBM 200 was 82.76% (95% CI 64.23–94.15) and 97.88% (95% CI 96.13–98.98), respectively, while the PPV and NPV of Hemocue was 70.83% (95% CI 48.91–87.38) and 96.43% (95% CI 94.34–97.91), respectively.

As shown in Table 2, the difference among the three methods of Hb estimation was found to be significant on application of Friedman's test ($p < 0.00001$). The Intraclass correlation coefficient of NBM 200 and SYSMEX KX-21 was 0.5099 [(single measures) (95% CI 0.4303–0.5800)] and 0.6754 [(average measures) (95% CI 0.6017–0.7342)], while that for Hemocue and SYSMEX KX-21 was 0.5315 [(single measures) (95% CI 0.4645–0.5922)] and 0.6941 [(average measures) (95% CI 0.6344–0.7439)]. The difference between the 3 methods was found to be statistically significant.

The concordance between the three methods was estimated using CCR. The CCR between NBM 200 and SYSMEX KX-21

Table 2 – Friedman's test.

Descriptive statistics						
	n	Minimum	25th percentile	Median	75th percentile	Maximum
SYSMEX	500	9.9	14.1	15	15.65	19.7
NBM 200	500	10.7	13.7	14.55	15.3	17.9
Hemocue	500	10.7	14.2	15	15.8	18.2
Friedman test						
F						45.2452
DF 1						2
DF 2						998
p						<0.00001
Multiple comparisons						
Variable	Mean rank		Different ($p < 0.05$) from variable nr			
(1) SYSMEX	2.057		(2) (3)			
(2) NBM 200	1.69		(1) (3)			
(3) Hemocue	2.253		(1) (2)			

was 0.5094 (bias correction factor/accuracy – 0.9605) while the CCR between Hemocue and SYSMEX KX-21 was 0.5310 (bias correction factor/accuracy – 0.9835).

The kappa value, which shows the agreement between the NBM 200 and SYSMEX KX-21, was found to be 0.746, thereby showing moderate agreement although statistically significant at $p < 0.0001$ as compared to 0.562 showing weak agreement although statistically significant at $p < 0.0001$ between Hemocue and SYSMEX KX-21.

Bland–Altman graphs were plotted in order to show concordance of the NBM 200 and Hemocue methods with the reference method SYSMEX KX-21 as well as between NBM 200 and Hemocue. This “difference plot” is a graphical method to compare two measurement techniques. This graphical method plots the differences (or alternatively the ratios) between the two techniques alongside the averages of the two techniques. On the other hand, the differences can be plotted against one of the two methods, if this method is “gold standard”. Horizontal lines are drawn at the mean difference, and at the limits of agreement, which are defined as the mean difference plus and minus 1.96 times the standard deviation of the differences. Both the Bland–Altman plots shown in Fig. 1A and B show outliers (i.e. beyond mean ± 1.96) and the values are distributed all over, meaning that the range of agreement is too wide.

Mountain plots were plotted to provide information about the distribution of the differences between methods. Mountain plots were plotted for comparison between NBM 200 and SYSMEX KX-21 (Fig. 2A), Hemocue and SYSMEX KX-21 (Fig. 2B), and finally for NBM 200 and Hemocue with SYSMEX KX-21 (Fig. 2C). All the plots confirmed that the two methods significantly differ from the reference method (SYSMEX KX-21).

Discussion

Hb measurement of whole blood or platelet donors prior to donation is an indispensable component of the donation process as it not only ensures that the required product is not collected from anemic donors, but it also ensures quality of the collected product. Donor comfort prior to, during, and after

donation plays a huge role in the donor recruitment as well as donor retention. Pain due to finger prick has been claimed as being the most fearful part of blood donation process.¹¹ Therefore, it is up to the blood transfusion services to make use of accessible noninvasive methods for predonation Hb screening of donors, but only after validating such devices.

Automated hematology analyzers have over years proved to have a higher precision and accuracy as compared to the other existing invasive Hb estimation methods. Therefore, SYSMEX KX-21 was taken as a reference method for evaluating other methods of Hb estimation.⁵ An ideal screening Hb estimation method is ought to be portable, painless, reasonably priced, and should have the ability to provide accurate results.¹⁰ Hemocue is one available technique used traditionally in the blood banks for predonation Hb screening as it offers the advantages of being simple, portable, yielding instant results, is battery operated, is accurate, dependable, and straightforward to use. However, one major drawback, especially in the case of apprehensive donors, is that it is “invasive”. This is where a pressing need for a noninvasive POCT Hb estimation method is felt, so that a potential platelet donor can be deferred from donation if he has low Hb level even before a venous sample is drawn for a complete blood count.

In our study, SYSMEX KX-21 detected 29 potential platelet donors out of 500 who had Hb value less than the mandatory 12.5 g/dl. Of these 29 ID, NBM 200 detected 22 (75.86%) whereas Hemocue detected only 17 (58.62%). This might have been due to the fact that since Hemocue uses capillary blood for Hb estimation, the values obtained are higher.¹² Thus, five ID would not have had to undergo a second prick to collect a venous sample for the readings to be obtained by SYSMEX KX-21, if only NBM 200 would have been used as a predonation Hb screening method before plateletpheresis. In a Korean study by Kim et al., NBM 200 detected 38.6% of ID, whereas Hemocue detected 54.3% of the ID.¹³ Observations by Singh et al. corroborated with our findings with respect to the precision of the methods; however, a study by Pinto et al. found Hemocue to be more precise than NBM 200.^{6,14}

A moderate agreement was found between values obtained by NBM 200 and SYSMEX KX-21 (as indicated by the kappa values), which could be due to the fact that a variety of factors,

Fig. 1 – (A) Bland–Altman plot comparing mean of SYSMEX KX-21 and NBM 200. (B) Bland–Altman plot comparing mean of SYSMEX KX-21 and Hemocue.

Fig. 2 – Mountain plots. (A) The curve is peaked over zero but has long tails (which indicate large difference leading to unacceptability of Hemocue as compared to SYSMEX KX-21). **(B)** The curve is peaked over zero but has long tails (which indicate large difference leading to unacceptability of the new method NBM 200, as compared to SYSMEX KX-21). **(C)** The combined mountain plot of SYSMEX KX-21 with Hemocue and NBM 200 shows peakedness above zero point; but at the same time; difference between the methods is also seen (different peaks for Hemocue and NBM200) and long tails indicating large differences between three methods. All these combined observations indicate that the methods differ from each other significantly.

viz. color of skin, thumb structure, local temperature, and also the donor position (sitting or standing), could have an effect on the Hb values.^{12,15} Nevertheless, the kappa values also suggested that Hb values obtained by NBM 200 were found to be more in agreement with SYSMEX KX-21 as compared to Hemocue 201+.

Hemocue 201+ was found to be more accurate and a slightly better technique as compared to NBM 200. However, the difference in coefficient of variation in the Hb readings obtained by these two methods was very minimal. So, although Hemocue 201+ appears to be statistically better than NBM 200, practically it may not be appreciable.

It is mandatory to obtain the complete blood count (CBC) of the potential platelet donor for which a venous sample of the donor is tested with the automated hematology analyzer. So, instead of deferring a platelet donor based on low Hb values after collecting the venous sample, it would be prudent to first obtain the Hb value by a noninvasive method. Only if the donor

qualifies for donation based on Hb criteria, we can then proceed and collect a venous sample for the CBC. If the Hb value is less than the mandatory 12.5 g/dl, as measured by the noninvasive method, the donor can be deferred from donation at this stage itself. This would avoid pricking the donors for a venous sample and then deferring them.

Our study was done on potential platelet donors vis-à-vis other studies conducted worldwide, which have been done on the whole blood donors. There has also been no consensus on the use of various equipments available for noninvasive Hb measurements in blood banking with respect to the whole blood donors.

To conclude, Hemocue 201+ was found to be more accurate whereas NBM 200 was found to be more sensitive, specific, precise, having a higher PPV and NPV, and more in agreement with the reference method as compared to Hemocue for predonation screening of Hb in plateletpheresis donors. Since a screening test ought to be more sensitive and specific, NBM

200 secures a distinct advantage over Hemocue 201+.⁶ Also, the prime benefit that it offers is that it is “noninvasive,” thereby assisting in retaining the valuable platelet donor pool, by improving donor comfort levels. In view of the above, we recommend performing the Hb estimation in potential plateletpheresis donors first by the noninvasive method, so as to avoid needless collection of a venous sample from the donor if the Hb is found to be less than 12.5 g/dl. The onus lies on the blood transfusion services all over the world to make use of appropriately validated gadgets that reduce the donor discomfort as efforts to recruit and retain donors will always fall short of the ever increasing demand of blood and blood components.

Conflicts of interest

The authors have none to declare.

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