Usefulness of non-invasive spectrophotometric haemoglobin estimation for detecting low haemoglobin levels when compared with a standard laboratory assay for preoperative assessment

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Background. Delay in diagnosis of anaemia during preoperative assessment poses logistic problems, leading to multiple clinic visits, inadequate preoperative management, and unnecessary delay of surgery. Therefore, we tested an instant spectrophotometric haemoglobin (SpHb) measurement technique to facilitate this assessment.

Methods. We evaluated portable instant SpHb vs standard laboratory screening of anaemia between March 2012 and December 2013. Paired Hb measurements were performed on 726 patients using SpHb (Pronto-7, Masimo Corporation, Irvine, CA, USA) and Hb measured on the same day using an automated analyser. The results were obtained from a group of 638 patients from the pre-anaesthetic clinic with expected normal Hb values, and 88 patients from the oncology clinic with known low Hb.

Results. Median (range) SpHb was 129.5 (67–171) compared with 136 g litre−1 (63–178) Hb measured using the automated system. Identifying Hb below a threshold of 130 g litre−1 for males had a high sensitivity (93%), while identifying a threshold of 120 g litre−1 for females had lower sensitivity (75%). The specificity for males (77%) and females (81%) was similar. Mean measurement bias and agreement: tolerability interval ratio was −8.1 g litre−1 and 2.78 for men and −3.1 g litre−1 and 2.44 for women.

Conclusions. SpHb was sensitive as a preliminary screening tool for detecting true low Hb values in males, but less sensitive in females. Instant SpHb measurement may enable prompt routine preoperative anaemia management, but its precision was lower than expected.

Clinical trial registration. This study is approved by the Tasmanian Human Ethics Committee, Australia and was registered prospectively in the Australian and New Zealand Clinical Trials Registry (http://www.ANZCTR.org.au/ACTRN12611001256965) and the World Health Organization Clinical Trials Registry (http://apps.who.int/trialsearch/trial.aspx?trialid=ACTRN12611001256965).

Keywords: laboratory Hb; pre-anaesthetic clinic; preoperative anaemia; preoperative screening; spectrophotometric Hb

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Haemoglobin (Hb) assessment is one of the most common laboratory tests performed, with an estimated 400 million tests yearly.1 2 The value of a reliable and convenient clinical method to measure Hb in patients who are undergoing major operative procedures3 is clearly established. An ideal method for clinical use should be easy to perform, accurate, reproducible, fast, and cost-effective.

The standard measurement of Hb involves either venepuncture, and to a lesser extent fingerprick, both of which are invasive methods that may cause pain and create a potential risk of infection for patients, and of needle-stick injury for clinical staff.1 2 In addition, the laboratory-based standard Hb test entails cost (transportation and processing) and some delay in the reporting of results. Using an instant non-invasive
point of care device reduces discomfort for patients and saves time in patient care, especially in certain clinical circumstances.\(^1\)\(^4\) Delays in the identification of patients with preoperative anaemia may delay both proper anaemia management and the operative procedure.\(^5\)

Masimo Pronto-7 (version 2.1.9, Masimo Corporation, Irvine, CA, USA) in conjunction with the Rainbow 4D Sensor or finger probe is a device that has been developed by the manufacturers for the non-invasive estimation of Hb. The technology involved is Rainbow signal extraction technology, or the spectrophotometric estimation of haemoglobin (SpHb), as it is now widely known.\(^6\)\(^7\)\(^8\)\(^9\)\(^10\) SpHb has the potential to overcome some of the shortcomings of invasive Hb sampling by enabling fast, accurate, needle-free Hb measurements.\(^8\)\(^9\)\(^10\)

The current study arose as a sub-study of a randomized controlled trial of i.v. iron infusion in patients identified as being anaemic before major surgery. The pilot component of this trial identified a logistical problem in the recruitment of patients after initial examination at the pre-anaesthetic assessment clinic, because only a minority of the patients found to be eligible were returning to the clinic for treatment of their anaemia, and therefore, the trial recruitment was poor and potentially biased. SpHb measurement was therefore undertaken in all patients as a screening process to identify potentially anaemic patients, and for recruiting the patients into the trial before they left the clinic.

**Objective**

The aim of the present analysis was to compare SpHb levels obtained non-invasively using the Masimo Pronto-7 device with Hb values from standard laboratory procedures (LabHb) using venous blood samples taken within 1 h of the SpHb measurement in two groups of patients.

**Patients and methods**

**Patient recruitment**

We conducted a parallel prospective assessment of SpHb vs standard Hb in 726 consecutive patients seeking care at the Launceston General Hospital (a tertiary referral teaching hospital in Tasmania, Australia) during the period of March 2012 to December 2013. Written informed consent was obtained from subjects taking part in a randomized controlled trial of i.v. iron infusion vs oral iron in patients identified as being anaemic before major surgical procedure. Of these, 638 patients had attended the pre-anaesthetic clinic (PAC) before major elective surgery and underwent SpHb in addition to routine laboratory testing that included standard Hb measurement. The SpHb acted here as a screening tool for the purpose of the study, clinical decisions for treatment were based on standard measurements (LabHb). The SpHb was performed by nine different registered nurses in the PAC.

**Haemoglobin measurement**

Elective surgery patients had their SpHb measured at the pre-anaesthetic check-up by a senior clinic nurse using the Masimo Pronto-7 device in an ‘average of three readings’ mode. This was followed by a standard laboratory blood test for Hb within 1 h. Those oncology patients who attended for routine laboratory Hb check had their SpHb measured at the time they had their blood collected. The non-invasive measurement by the SpHb technique and the blood collection occurred within ~1 h of each other for all patients. SpHb was performed by nine different registered nurses in the PAC.

Fresh ethylene-diaminetetraacetic acid blood specimens were collected prospectively from all patients and were analysed within 2 h of collection as per routine laboratory turnaround time. The Hb was measured at a National Association of Testing Authorities accredited laboratory using a Sysmex XE\(^5\)5000\(^TM\) automated haematology analyser (Sysmex Corporation, Kobe, Hyogo, Japan).

The lower limit of normal Hb was defined as 130 g litre\(^{-1}\) for men and 120 g litre\(^{-1}\) for women as per routine laboratory procedures. As the coefficient of variation (CV) for the laboratory Hb is routinely performed on quality control material, and as there is no equivalent QC material or process for SpHb measurements, a separate procedure was performed to generate the necessary data. The CV for the SpHb measurement was estimated by performing 30 repeated estimates of Hb over 90 min on each of seven laboratory and research staff who were not subjects in the trial (four females: LabHb 83, 100, 113, and 146 g litre\(^{-1}\); three males: LabHb 81, 117, and 151 g litre\(^{-1}\)). The CV for the laboratory Hb assay was estimated by 10 repeated assays on single blood samples from each of 6 persons (four females: LabHb 86, 103, and 123 g litre\(^{-1}\); three males: LabHb 84, 106, and 128 g litre\(^{-1}\)).

**Statistical methods**

Patient characteristics were compared using the T-test (age, BMI standard, and LabHb) and where distributions were strongly non-normal: oxygen saturation, pulse rate, and perfusion index (PI), ordered logistic regression was used.

Two assessments were performed: (i) on assessment of the utility of the SpHb device as a screening tool to identify low Hb levels in men (<130 g litre\(^{-1}\)) and women (<120 g litre\(^{-1}\)). Sensitivity, specificity, and receiver operating characteristics (ROC) area were estimated using ROC analysis, (ii) a Bland–Altman assessment of the two assay methods as recommended by Mantha and colleagues\(^11\); repeatability was expressed as the coefficient of variation (CV: calculated as SD/mean) for each test method; the SpHb-laboratory difference between method result pairs of measurements (y-axis) were plotted against the mean value of the two methods (x-axis);
the association of SpHb to laboratory difference and mean Hb values (mean of laboratory and SpHb using the standardized normal transformation subject minus mean minus of mean divided by $sd \text{ of mean}$); $sd$ of the laboratory to SpHb difference and 95% confidence intervals (CIs) of that difference; and finally, prior clinically relevant limits of agreement were set as $\pm 10 \text{ g litre}^{-1}$ for $\pm 2 \text{ sd}$ in this case.

The ratio of observed agreement and prior tolerable limits of agreement (agreement: tolerability index ratio (ATI ratio)) were calculated. Also the effects of gender, oxygen saturation ($O_2 \text{sat}$), pulse rate, and PI were estimated by multiple mixed effects linear regression. The component values for the CV and the association of SpHb-laboratory difference and mean Hb values were estimated using mixed effects linear regression. All analyses were performed using Stata 13.1/MP2 (StataCorp, College Station, TX, USA).

**Results**

A total of 726 patients were initially recruited, 638 from the pre-anaesthetic clinic and 88 from the oncology clinic. However, 27 patients did not proceed to have a blood sample collected for a laboratory-based Hb and were excluded. The patient characteristics and LabHb of the 699 enrolled patients are given in Table 1. There was a 20% failure rate in obtaining a technically satisfactory SpHb reading, leaving 584 patients. We noticed an initial higher failure rate that was possibly attributable to the use of an inappropriate sized finger probe, this however, improved when two sizes of probe became available. We also noticed that patients with Raynaud’s or other conditions resulting in poor peripheral circulation failed to register a reading. The PI, a quantitative measure of perfusion, was significantly lower in women (Table 1).

In the 584 patients analysed, median SpHb was 129.5 (67 – 171) compared with 136 g litre$^{-1}$ (63 – 178) for LabHb.

Correct classification of patients into ‘normal’ or ‘low’ Hb level in the oncology group with a high rate of low laboratory Hb was obtained in 95% of men and 91% of women. However, in the PAC group where low Hb levels were less common, it was 79% for males and 78% for females (Table 2). For all patients, correct classification was obtained in 81% of men and 80% of women. In the PAC patients, using the threshold of 130 g litre$^{-1}$ for men, sensitivity was 92% for detecting normal Hb levels, while using the threshold of 120 g litre$^{-1}$ for women, sensitivity was lower (57%). The specificity was 74% in men and 82% in women. This is demonstrated graphically in Figure 1. Thus, the overall performance of the SpHb measurement to identify low Hb levels was significantly lower in women (ROC area difference $P=0.002$).

**Bland–Altman pair-wise comparison**

The coefficient of variance for mean SpHb was 4.5% (CI: 3.6–5.2%), while the LabHb between sample CV was 0.57% (CI: 0.46–0.68%). Thus, the SpHb measurement had a much larger CV than the laboratory Hb measurement. The difference appeared to be roughly even across the range of Hb values. However, there was a large range of differences between the individual subject SpHb and laboratory Hb measurements, and the observed limits of agreement were substantially greater than the a priori limits set for this comparison. The plot of difference (SpHb to LabHb) vs mean Hb is shown in the lower portion of Figure 2A and B. Mean difference was $-5.6 \text{ g litre}^{-1}$ (SD: 13.1) in all 584 patients, $-8.1 \text{ g litre}^{-1}$ (SD: 12.8) in the 294 men and $-3.1 \text{ g litre}^{-1}$ (SD: 12.9) in the 290 women.

The age-adjusted regression coefficient for the association between the difference and mean Hb in the PAC patients was $-0.28 \text{ g litre}^{-1}$ per SD (18 g litre$^{-1}$) of mean Hb (CI: $-2.15-1.60; P=0.77$) in men and $1.34 \text{ g litre}^{-1}$ per SD (18 g litre$^{-1}$) of mean Hb (CI: $-1.06$ to 3.73; $P=0.27$) in women. No significant differences between the regression coefficients were found for men and women in the PAC and oncology clinics, and the estimates were not altered by adjustment for age, gender or clinic type. In the PAC group, the mean difference (SpHb to LabHb) adjusted to the mean Hb concentration of 133 g litre$^{-1}$ was $-9.7 \text{ g litre}^{-1}$, with a $\pm 2 \text{ sd}$ range from $-37.5 \text{ (CI: -40.9 to -34.9)}$ to 18.0 (CI: $15.5-21.5$) for men, and $-4.5 \text{ g litre}^{-1}$, with a $\pm 2 \text{ sd}$ range from $-28.9 \text{ (CI: -31.9 to -26.6)}$ to 19.9 (CI: $17.6-22.9$) for women.

The prior limits of agreement were set as $\pm 10 \text{ g litre}^{-1}$ for $\pm 2 \text{ sd}$. Thus, the actual limits of agreement exceeded the prior limits by a large margin: $\pm 27.8 \text{ g litre}^{-1}$ (agreement:tolerability

| Table 1 | Patient characteristics. *The number of patients seen in pre-anaesthetic and oncology clinics (all), and number of those patients who had a technically successful estimate of SpHb. †Comparison of variables between all patients and patients with successful SpHb measurements in men and women separately. ‡Comparison of variables between men and women in patients with successful SpHb measurements. |
|---------|-------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|         | Men (n=354) | SpHb* (n=294) | P-value†        | Women (n=345)  | SpHb* (n=290) | P-value‡       | P-value‡       |
| Age     | 65.6 (12.0) | 65.5 (11.7)    | >0.90           | 61.1 (14.9)    | 61.4 (14.7)    | 0.80           | <0.001         |
| BMI     | 28.3 (5.4)  | 28.5 (5.4)     | 0.64            | 29.3 (6.8)     | 29.2 (6.8)     | 0.85           | 0.18           |
| Laboratory Hb | 138 (21) | 138 (22)      | >0.90           | 129 (18)       | 129 (18)       | >0.90           | <0.001         |
| O2 saturation | 96.3 (2.3) | 96.9 (2.2)    | <0.001          | 76.3 (13.1)    | 76.3 (13.1)    | <0.001          | <0.001         |
| Pulse rate | 72.3 (13.4) | 4.93 (3.66)   | <0.001          | 76.3 (13.1)    | 76.3 (13.1)    | <0.001          | <0.001         |
| Perfusion index | 6.32 (6.57) | 6.32 (6.57) |              | 6.32 (6.57)    | 6.32 (6.57)    |              |               |
interval ratio: 2.78) for men and ±24.4 g litre⁻¹ (ATI ratio: 2.44) for women.

There was no consistent effect of PI in men, but the PI was associated with a more positive difference in women (1 SD PI was associated with a 3.2 g litre⁻¹ change; CI: 1.0–5.3; \(P=0.004\)). In addition, it was found that in women, the measurement difference (SpHb to LabHb) was associated with the PI (1 SD increase in PI was associated with a 3.96 increase in difference; CI: 1.8–6.13; \(P<0.001\)), while no such association was seen in men (0.29; CI: −0.81 to 1.4; \(P=0.60\); for women vs men \(P=0.003\)). Furthermore, in women PI was associated with mean Hb ((SpHb−LabHb)/2) (1 SD increase in mean Hb was associated with a 0.81 increase in PI; CI: 0.12–1.49; \(P=0.021\)), while no such association was seen in men (−0.20; CI: −0.75 to 0.35; \(P=0.48\); for women vs men \(P=0.025\)).

**Discussion**

We found that SpHb was useful in the identification of male patients with low Hb, but was less accurate in identifying low Hb levels in females. In 20% of patients, technical failures prevented a successful SpHb reading. The precision of the SpHb was substantially poorer than that of the standard laboratory method. We found that SpHb was satisfactory as a method for screening the patients attending the pre-anaesthetic clinic to accelerate and facilitate further assessment of patients with possible preoperative anaemia. This might include further testing with iron studies and routine blood tests. For the majority of patients, the advice given after the immediate availability of SpHb at the first visit was not changed by subsequent measurement of laboratory Hb using the standard technique, but in a minority the advice did change.

The reason for the differences found between women and men in terms of the usefulness of SpHb as a screening tool is unclear, but may be related to the relationship with PI seen in women but not men. It is also possible that the SpHb device was optimized for men rather than women, or additional factors may be involved. Perfusion index has been reported to be unresponsive to electrical stimulation in women more than 60 years but not men. Since in our study, 68% of

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**Table 2** Comparison of performance of SpHb with simultaneous laboratory Hb. *Sensitivity, specificity, correct classification, and receiver operating characteristic (ROC) curve area estimated using the non-parametric method (Stata13.1), using a classification for low Hb concentration of <130 g litre⁻¹ for men and 120 g litre⁻¹ for women as measured by the laboratory. This analysis assumes that the SpHb Hb test is being used to identify patients with possibly low Hb who will subsequently be managed using laboratory Hb analysis. †Comparison of the ROC area: all \(P=0.019\); PAC \(P=0.004\); ONC \(P=0.19\).
women were more than 55 years and 62.5% were more than 60 years, this may offer an explanation for the difference between men and women seen here.

Measurement of SpHb is not as rapid or straightforward as measuring SpO2. Ambient light, patient movement, irregular heart rhythm, poor peripheral circulation, and operator inexperience may all have an effect on the success rate of obtaining an SpHb reading. In this study, we used the ‘best of three readings’ mode of the Masimo Pronto-7 device. The inverse association between SpHb/Laboratory difference and LabHb value is consistent with a regression-to-mean phenomenon which is consistent with a significant amount of signal noise in the SpHb measurement. These difficulties are likely to have contributed to the substantial difference in the precision of the SpHb data (mean %) compared with the CV 4.5% standard method laboratory Hb (mean % CV 0.57%).

The positive association between SpHb/Laboratory difference and PI indicates that less Hb is detected when the blood flow through the finger is impaired. A digital nerve block during surgery has been found to improve the accuracy of non-invasive Hb monitoring and facilitates the use of SpHb as a guide to transfusion decisions. The hypothesis is that increasing perfusion to the finger improves the accuracy of Hb monitoring. In our cohort, finger perfusion, as measured by the device’s PI, was lower in females.

We included a group of ONC patients to test the utility of SpHb in detecting known low Hb. It did this successfully, with somewhat better sensitivity and specificity than that obtained in PAC patients, in whom the range of different Hb levels was greater. Our study showed that SpHb was able to identify low laboratory Hb values in male PAC patients (<130 g litre$^{-1}$), such that fewer than 10% of male patients with low Hb levels were missed. The performance of SpHb was less satisfactory in women in the PAC, where ~40% of low Hb levels (<120 g litre$^{-1}$) were not accurately identified.

The utility of point of care testing of Hb is accepted in earlier studies as significantly reducing the time taken to make patient management decisions which are dependent on blood results. Over recent years, there have been further refinements in the technology of continuous non-invasive Hb monitoring that resulted in the technology of the Masimo Pronto-7 SpHb used in this study. A couple of studies have described comparison of both the earlier model of a continuous non-invasive Hb monitor and Radical-7 and Pronto-7, with standard laboratory analysers. It is worth noting that the study published by Raikhel assessed the accuracy of non-invasive vs invasive point of care testing of Hb. There was a 2.5% failure rate in the non-invasive measurement (using Pronto-7) whereas the invasive technique (HemoCue) was successful in all subjects. The non-invasive and invasive techniques had similar bias and variance.

Masimo Pronto-7 Hb measurements have been shown to correlate moderately well with laboratory analyser Hb results, especially in men, but precision remains an issue. If the microcirculation were the same as the macrocirculation we could expect blood sampling at either site to be equivalent. However, this was reported previously as discordant, which may explain the difference between invasive estimation from the veins or artery (macro-circulation) and both invasive and non-invasive from the peripheries (microcirculation and macro-circulation). Perhaps further refinement of the technique and the algorithm could in future remove the small (but significant) differences in the two results, and there are several research projects underway to make more suitable devices. The introduction of an in vivo adjustment/synchronization feature in the Masimo Radical-7, is a step in the process of further refinement. The results from the Masimo Pronto-7 come close to the Hb results obtained from the macrocirculation (by laboratory analyser).

SpHb could be a useful screening tool in the PAC that would allow faster diagnosis of anaemia enabling detailed examination, further investigation, and management at the time of initial clinic visit. This may obviate the necessity of patients being recalled on the next day after a laboratory result becomes available, which creates obstacles for those who live distantly from the hospital. Undertaking surgery in patients with anaemia is associated with poorer outcomes and higher transfusion rates. Treatment for iron deficiency, the most common cause of anaemia, is readily available in the form of oral and parenteral iron with or without erythropoietin. Being able to start the appropriate treatments at the initial PAC visit would be more convenient and effective for patients. However the imprecision of SpHb demonstrated here suggests that treatment decisions should ultimately be determined by LabHb measurements.

SpHb screening should work well in men where SpHb has a low ‘false normal’ rate but further work may be required to establish whether SpHb is accurate enough to do the same for women. Future options for additional clinical indications include: urgent Hb measurement in Emergency Departments, in-patient transfer situations, intraoperatively, in the intensive care unit and as a screening tool before blood
Fig 2  (A and B) Relationship between laboratory and SpHb estimates of Hb concentrations in preoperative patients (A), and all patients (B).
Furthermore, it has huge potential in paediatric populations where invasive Hb is less acceptable. Using a specific paediatric probe may help overcome fears of children and parents requiring or undergoing Hb testing. SpHb would also be ideal for patients who suffer from needle phobia. However, proper validation of SpHb technology in these subgroups of patients is required.

Our data suggest that SpHb has a reasonably good ability to identify male patients with low Hb, but is less effective at identifying low Hb levels in females. Instant non-invasive SpHb measurement has the potential to be integrated into a routine preoperative work-up in the PAC to identify patients with low Hb and hence offers the option of prompt management of preoperative anaemia. Nevertheless, the precision of SpHb is within the unacceptable range. This precision would be too low to base clinical decisions concerning treatment, such as the initiation of iron therapy in anaemic patients. Patients would also need to be informed about such factors if SpHb measurements are to be used as a screening tool.

Authors’ contributions
A.A.K.: organized and coordinated all aspects of the research including study concept and design, and writing, reviewing, editing and approving the manuscript. C.R.C., M.V., and I.K.R.: contributed to the study design, analysis and interpretation of data, and writing reviewing and approving the manuscript. M.T., C.M.C., and M.S.: collected and analysed the data, performed the procedures, drafted the article, and finally approved the manuscript.

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Declaration of interest
All authors declare no conflict of interest in relation to this research. There are non-financial associations that may be relevant or seen as relevant to this research.

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References
8 Aoyagi T, Miyasaka K. The theory and applications of pulse spectrophotometry. Anesth Analg 2002; 94: 593–5
17 Lange KH, Jansen T, Asghar S, Kristensen PL, Skjonnemand M, Norgaard P. Skin temperature measured by infrared thermography after specific ultrasound-guided blocking of the musculocuta-neous, radial, ulnar and median nerves in the upper extremity. Br J Anaesth 2011; 106: 887–95
19 Shah N, Martinez GJ, Osea EA. Accuracy and precision of the Pronto-7 for non-invasive hemoglobin testing in an outpatient setting. Blood 2011; 118: 3147


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