PATIENT BLOOD MANAGEMENT

PERIOPERATIVE BLOOD LOSS: ESTIMATION OF BLOOD VOLUME LOSS OR HAEMOGLOBIN MASS LOSS?

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Background - Perioperative blood loss is an essential parameter in research into Patient Blood Management. However, currently there is no "gold standard" method to quantify it. Direct measurements of blood loss are considered unreliable methods, and the formulae to estimate it have proven to be significantly inaccurate. Given the need for better research tools, this study evaluated an estimation of haemoglobin mass loss as an alternative approach to estimate perioperative blood loss, and compared it to estimations based on blood volume loss.

Material and methods - We studied one hundred consecutive patients undergoing urological laparoscopic surgery. Both haemoglobin mass loss and blood volume loss were directly measured during surgery, under highly controlled conditions for a reliable direct measurement of blood loss. Three formulae were studied: 1) a haemoglobin mass loss formula, which estimated blood loss in terms of haemoglobin mass loss, 2) the López-Picado's formula and 3) an empirical volume formula that estimated blood loss in terms of blood volume loss. The empirical volume formula was developed within the study with the aim of providing the best possible estimation of blood volume loss in the studied population. The formulae were evaluated and compared by assessing their agreements with their respective direct measurements of blood loss.

Results - The haemoglobin mass loss formula met the predefined agreement criterion of ±71 g, with 95% limits of agreement ranging from 0.6 to 44.1 g and a moderate overestimation of 22.4. In comparison to both blood volume loss formulae, the haemoglobin mass loss formula was superior in every agreement parameter evaluated.

Discussion - In this study, the estimation of haemoglobin mass loss was found to be a more accurate method to estimate perioperative blood loss. This estimation method could be a robust research tool, although more studies are needed to establish its reliability.

INTRODUCTION

Perioperative blood loss is an essential parameter in research into Patient Blood Management, especially in the evaluation of strategies aimed at reducing perioperative bleeding such as pharmacological interventions, anesthetic management and surgical techniques.

Additionally, in comparison with other clinical outcomes (e.g., transfusion rates), blood loss could provide more robust evidence, as it might be less influenced by confounding factors and could, therefore, be more easily compared among trials. However, blood loss quantification remains unreliable and inaccurate. It is well known that direct measurements do not ensure trustworthy values, particularly in surgical interventions that involve the use of gauzes or hidden losses (e.g., orthopaedic surgery). Given the need for reliable quantification, it has been suggested that estimation formulae based on anthropometric and haematological parameters could be more useful approaches, as they overcome the limitations of direct measurements. Nevertheless, current estimation formulae have proven to be significantly inaccurate.

Despite the lack of a "gold standard" method, blood loss is still being used in many clinical studies as a valid outcome.

We hypothesised that the estimation of haemoglobin mass loss could be more accurate than the traditional estimation based on volume units. Considering that, during surgery, several variables constantly modify the volume of circulating blood, causing it to shift among a wide range of haemodilution states, hence the blood lost during this period could have variable degrees of dilution. Additionally, blood loss may occur at different time points during surgery and, therefore, during different haemodilution states. Consequently, the actual blood volume loss might not be correctly ref lected in the haematological parameters used by formulae, and thus, it might not be accurately estimated. Conversely, an estimation of haemoglobin mass loss would be based exclusively on the lost blood content, avoiding potential errors related to the degree of blood loss dilution.

This study was designed **as a first proof of concept** for the previously proposed hypothesis. For this purpose, a formula based on haemoglobin mass was constructed, and then evaluated and compared to estimation formulae based on blood volume loss, in a highly controlled scenario. The formulae were evaluated and compared by assessing their agreements with their respective direct measurements of blood loss, and by means of a comprehensive analysis of the statistical agreements.

MATERIAL AND METHODS

Study design

This study was carried out using the same protocol and methods that were described in a previous study, but extended with a direct measurement of haemoglobin mass in the lost blood.

Since this study was **designed as a first "proof of concept"** in order to assess the accuracy of the formulae to estimate perioperative blood loss, we decided to avoid any possible confounding factor and thus performed the study in highly controlled conditions. Cases of postoperative bleeding and or those in which gauzes were used during surgery were excluded with the intention of improving the reliability of the measured blood loss values. Along the same lines, cases of red blood cell (RBC) transfusions during the perioperative period were also excluded. Urological procedures were chosen since significant blood loss was expected.

Therefore, **the exclusion criteria** were as follows:

1) suspected or confirmed coagulopathy,
2) requirement for surgical gauzes during surgery, including conversion to open surgical techniques,
3) transfusion of RBCs during the perioperative period,
4) significant postoperative bleeding (>50 mL in surgical drains, gross haematuria or any other type of blood loss).

Perioperative care and measurements

General anaesthesia was performed in all cases. **The intraoperative fluid protocol consisted of both maintenance therapy (1 mL/kg/h of crystalloid) and replacement of blood loss (1 mL of crystalloid for each mL of directly measured blood loss).** If necessary, vasoactive agents were titrated to obtain a mean blood pressure >65 mmHg. An intraperitoneal surgical drain and a urinary catheter were placed in all patients and withdrawn 48 hours after surgery. **Postoperative fluid therapy consisted of crystalloids (20 mL/kg/day).** Fluid balance was registered by an electronic recorder (PuputTM; IPA, Barcelona, Spain), which calculated total fluid balance, taking into account fluid administration, blood volume loss and urinary output.

Since different methods and units of quantification were used, we defined:

- measured blood volume loss (VMBL): direct measurement of blood loss in volume units (mL);
- measured haemoglobin mass loss (mHbMBL): direct measurement of haemoglobin mass in the lost blood in mass units (g).

Both direct measurements of VMBL and mHbMBL were used as the reference values in the evaluation of their respective estimation formulae.

- Estimated blood volume loss: estimation of blood loss in volume units, calculated either by López-Picado's formula or by the empirical volume formula (VEBL);
- estimated haemoglobin mass loss (mHbEBL): estimation of haemoglobin mass loss, calculated by the haemoglobin mass loss formula described later.

Measurement of blood volume loss

The whole suction system was **heparinised** before surgery (25,000 IU of heparin in saline solution) and a continuous flow of saline solution prevented any clotting of blood loss. The required volumes of heparin and saline solutions were carefully recorded. Intraperitoneal residual losses were suctioned through surgical drains into a canister at the end of the surgical procedure. The total volume contained in the canister was measured after the end of the surgical procedure by a system capable of determining differences up to ±10 mL. VMBL was determined by subtracting the added fluids (heparin and saline solutions) from the total volume contained in the surgical canister.

Measurement of haemoglobin mass loss

Haemoglobin mass loss was assessed in the lost blood using the spectrophotometric method. After agitation to ensure mixing, two samples were drawn from the suction canisters at the end of the surgical procedure.

The haemoglobin concentration in the samples were measured by a spectrophotometric haemoglobin analyser.

The haemoglobin concentration values of the two samples were averaged to obtain a final value. In cases where significantly different values were obtained **(defined by a difference >0.4 g/ dL between the samples**), a third sample was drawn, and the average of the three samples was considered for further calculations. HbMBL was determined by multiplying the haemoglobin concentration of the canister-derived samples by the total volume contained in the canisters.

The HemoCue® system was chosen to assess the haemoglobin concentration of the content of the suction canisters because it is a validated method for this purpose15. It has a significant accuracy (95% limits of agreement between −0.39 and 0.36 g/dL)15 and it corrects for turbidity of the samples. This feature was considered to be of utmost importance, since most laboratory haemoglobin analysers do not correct for turbidity, and surgical blood loss is often contaminated with other fluids (e.g., lipids). The measurement of haemoglobin mass in the lost blood (as described above) has been considered by other authors as a reliable method in research.

Haemoglobin mass loss estimation formula

As in other blood loss formulae, the haemoglobin mass loss formula was conceived on the basis that blood loss can be estimated using three parameters: **patient's estimated blood volume, pre- and postoperative haemoglobin concentrations, and also considering a euvolaemic state between the pre- and postoperative time points at which the patient's haemoglobin concentration is measured.**

In currently used blood loss estimation formulae, blood loss (in volume units) is calculated by multiplying the perioperative difference of haemoglobin (or haematocrit) by the patient's estimated blood volume. The main difference between these formulae is that the perioperative difference of haemoglobin (or haematocrit) is adjusted by different mathematical functions, which intend to correct for the expected dilutional effect caused by blood loss.

Nevertheless, the assumption of a predictable dilutional effect is unlikely to be correct in the perioperative setting since during this period, significant unpredictable inter-and intraindividual variations of blood volume (more specifically, plasma volume) have been demonstrated9-13, which have been attributed to the complexity of the physiological volume replacement mechanisms.

Additionally, ongoing fluid administration, pathological fluid shifts and anaesthesia itself also affect the plasma volume status[9,10]. Since plasma volume varies through the intra- and perioperative periods, blood volume shifts between a wide range of haemodilution states. As blood loss usually occurs at different time points during surgery, the blood lost during this period may have different degrees of dilution, and therefore, different haemoglobin contents. Consequently, blood volume loss would not be correctly ref lected in the haematological parameters used in the blood loss estimation formulae.

Regarding haemoglobin mass (or RBC mass), it has been observed that the circulating RBC mass also varies during the intraoperative period. Such variations could be explained by the blood loss itself, but also by acute redistributions between the circulating and non-circulating blood compartments19. However, it has also been observed that the redistribution effect may only occur during the intraoperative period, and that the circulating RBC mass remains relatively stable during the rest of the postoperative period19. Therefore, using haemoglobin mass as the unit to estimate blood loss may be more robust than the volume approach. This notion has also been suggested by previous investigations that found a substantial relationship between the lost RBC mass and perioperative haematocrit differences19, whereas weak or no significant relationship has been found between the differences in blood volume loss and perioperative haematocrit.

However, despite the unpredictable behaviour of perioperative volume status, previous studies have observed that blood volume **is normal** after preoperative fasting22 and that blood volume may be restored to preoperative values by the time the patient reaches the nadir (lowest) haematocrit or haemoglobin concentration19,20. In addition to these lines of evidence, circulating haemoglobin mass (as for RBC mass) has been observed to be adequately estimated considering the patient's blood volume (only in euvolaemic conditions) and haemoglobin concentration23.

Therefore, loss of haemoglobin mass might be estimated by multiplying the perioperative difference in the patient's haemoglobin concentrations and the patient's blood volume. The resulting formula (haemoglobin mass loss formula) is summarised as:

$$\mathbf{mHbEBL = 100 \times (Hbpreop - Hbnadir) \times BV}$$

where mHb_{EBL} (g) is the estimated haemoglobin mass loss, Hb_{preop} (g/dL) is the patient's preoperative haemoglobin concentration, Hb_{nadir} (g/dL) is the patient's lowest postoperative haemoglobin concentration, and BV (mL) is the patient's estimated blood volume, calculated using the ICSH formula

Blood volume loss formulae

To correctly evaluate the accuracy of the haemoglobin mass loss formula, two formulae for estimating blood volume loss were tested

1. ***The López-Picado's formula***. This formula was chosen since it had previously been shown to achieve better agreement with directly measured blood loss than other blood volume loss formulae7. The details of this formula are specified in the *Online Supplementary Content*.

2. ***Empirical volume formula***. An empirical formula was developed with the intention of providing the best possible estimation of blood volume loss in the study population. Since it was both developed and tested using the study's population data, it was not treated as an actual formula to be considered in other populations. Therefore, it was used only as a tool to compare the performance of the haemoglobin mass loss formula. The formula was used to calculate the VEBL

Statistical analysis

Categorical data were expressed as frequencies and percentages. Variables were analysed using the Shapiro- Wilk test to determine whether data were normally distributed. Quantitative data were expressed as means and standard deviations (SD) if they were normally distributed or medians and interquartile ranges (IQR) if they were not.
The evaluation of the estimation formulae was performed by contrasting its achieved agreements with their respective measurement of blood loss. The mHb_{EBL} was evaluated considering its agreement with the mHb_{MBL}, while the estimates of the López-Picado's formula and the V_{EBL} were evaluated considering their agreement with the V_{MBL}.

The statistical analyses performed in this study assessed different parameters and aspects of the agreement between each estimation formula and the respective blood loss measurement.
We assessed:

1) absolute agreement as evaluated in the Bland-Altman analysis;
2) the agreement's strength, accuracy and precision, as evaluated by the concordance correlation coefficient (CCC) analysis;
3) the agreement's consistency over different amounts of blood loss.

1. **Bland-Altman analyses** were used to determine the absolute agreement per formula (in their respective units of measurement). A unit conversion between haemoglobin mass loss and blood volume loss was set to compare the agreement achieved by the formulae, albeit only in a rough approximation. In this study, 71 g of haemoglobin mass loss and 560 mL of blood volume loss were considered to be equivalent since they respectively correspond to approximately 10% of the total haemoglobin mass and 10% of the total blood volume of an average non-anaemic adult. These values were also used to define the agreement's criterion for an acceptable absolute agreement. Bland- Altman plots and histograms of differences (between estimated and measured values) were constructed to visualise and interpret data.

2. **The concordance correlation coefficient (CCC)** was chosen as the main statistical method to compare the agreement achieved between each formula and the respective measurement of blood loss. In contrast to the Bland-Altman analysis, the CCC provides an evaluation of the agreement that is independent of the measurement unit, thus allowing a proper comparison of the overall performance of the formulae. Agreement is evaluated in terms of precision (ρ), accuracy (Cb) and the agreement's strength (ρc: concordance correlation coefficient, or CCC index). In this study, the CCC parameters were used only to compare the agreement of the formulae. No minimum acceptable values were considered because there was no universal interpretation of the values.

3. The agreement's consistency for each formula was assessed to determine their agreement among different amounts of blood loss. The consistency of the agreement was evaluated in Bland-Altman plots on percentage differences by visually analysing the dispersion width of the estimates at different amounts of blood loss. Considerable consistency was defined when the dispersion width in the Bland-Altman plot became narrower at greater magnitudes of blood loss.

Sample size determination

Based on data from a preliminary analysis of the differences between the measured and estimated haemoglobin mass loss, it was felt that a sample of 100 cases was required. This calculation was performed according to the method proposed by Lu *et al*.[30] to determine the necessary sample size to observe maximum 95% limits of agreement (LOA) for ±71 g in the Bland-Altman analysis. Sample size was calculated with a statistical power of 90% and a level of significance of 0.05 (two-tailed).

Sample size was calculated without considering the CCC analysis since no minimum acceptable value was predefined.

RESULTS

One hundred and twelve patients were enrolled. Of these, twelve were excluded, as they met exclusion criteria (4 patients were transfused, 6 had postoperative bleeding, and 2 were converted to an open surgery technique or required the use of gauzes). The data obtained in the remaining 100 patients are summarised in **Table I**. Only 3 patients required a third measurement of haemoglobin mass loss as it was defined in the study design. Patients' nadir haemoglobin concentrations were reached 48 hours after surgery in 95 subjects, while 3 and 2 patients reached the nadir 24 hours and 72 hours after surgery, respectively.

The mHb_{MBL} r anged f rom 0 .6 t o 2 72.8 g (median: 44.5 g; IQR: 19.3-89.2). The V_{MBL} ranged from 50 to 2,050 mL (median: 640 mL; IQR: 260-870). Due to the non-normal distribution of the mHb_{MBL} and the V_{MBL} d ata, h istograms w ere c onstructed f or each variable (**Figure 1A** and **B**).

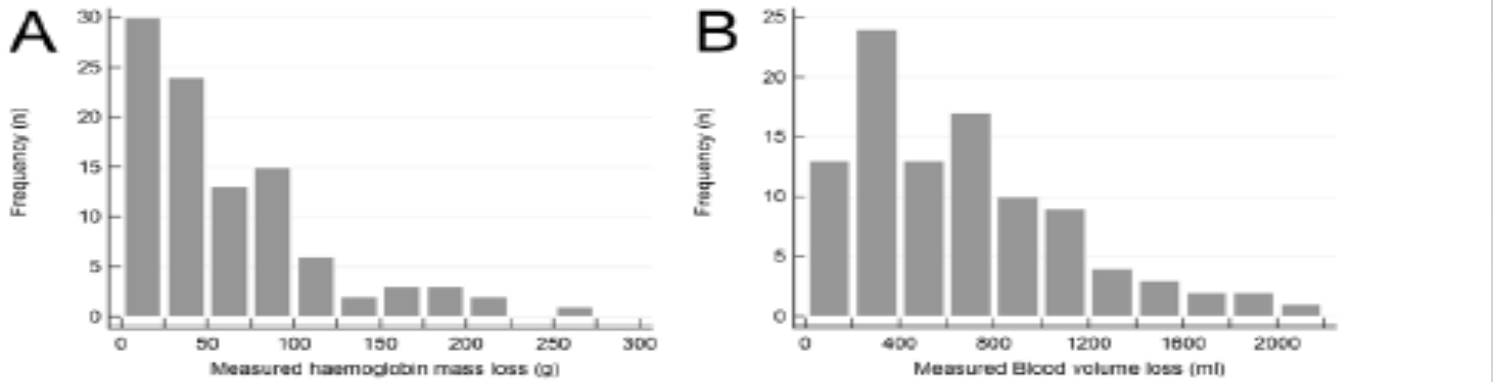

Figure 1 - Histograms of measured haemoglobin mass loss (Hb_{MBL}) (A) and measured blood volume loss (V_{MBL}) (B).
The amount of each parameter (x-axis) is shown in ranges. Significant blood volume loss was observed: bleeding was = 500 mL in 54% of patients, 1,000 mL in 18% of patients, and 1,500 mL in 7% of patients.

Table I - *Patient characteristics (n=100)*

Age (yrs), mean (range)	61 (39-82)
Sex ratio, male/female, n	77/33
Height (cm), mean (SD)	168 (8)
Weight (kg), mean (SD)	76 (14.0)
BMI (kg/m²), median (IQR)	27.3 (24.1-29.3)
ASA physical status classification, n	
1	16
2	67
3	17
Estimated intravascular blood volume (mL) (ICSH formula), mean (SD)	5,448 (719)
Total fluid balance (mL) (mean [SD; range])	452 (622; −1,058-2,285)
Surgical intervention, n	
Robotic prostatectomy	43
Laparoscopic partial nephrectomy	35
Laparoscopic radical nephrectomy	15
Laparoscopic nephroureterectomy	7
SPatients' perioperative haemoglobin concentrations	
Preoperative haemoglobin concentration (g/dL), mean (SD)	14.4 (1.6)
Postoperative (nadir) haemoglobin concentration (g/dL), mean (SD)	11.8 (1.5)

ASA: American Society of Anesthesiologist physical status classification; BMI: body mass index; ICSH: International Council for Standardization in Haematology; IQR: interquartile range; SD: standard deviation.

Absolute agreement

The Bland-Altman analysis of the haemoglobin mass loss formula resulted in a bias of 22.7 g (95% confidence interval [CI]: 20.6-24.7), an upper limit of agreement of 42.8 g (95% CI: 39.3-46.3) and a lower limit of agreement of 2.5 g (95% CI: −0.9-6.0), remaining between the ±71 g predefined limits of agreement. Although the formula over-estimated blood loss, this was considered to be moderate, as it represented approximately 3.2% of the total haemoglobin mass of an average healthy adult. Using the same unit conversion, the haemoglobin mass loss formula approximately estimated 20 to 338 mL of blood volume loss of an average healthy adult. In contrast, blood volume loss formulae exceeded the ± 560 mL limits of agreement defined and showed inferior agreements with the measured blood volume loss, as seen in the histograms of differences. Bland- Altman plots and histograms of differences (between estimated and measured values) per formula are displayed in **Figure 2**.

A subanalysis not included in the initial design was performed regarding the effect of total fluid balance on the agreement of the haemoglobin mass formula. Total fluid balance was calculated per patient as the cumulative balance immediately before surgery until the time in which the lowest (nadir) haemoglobin concentration was reached. Total fluid balance was divided by the population's mean into two groups: small or negative balances (<452 mL) and large balances (≥452 mL). No substantial difference was found between the small or negative balances group (LOA: 3.7-41.6) and the large balances group (LOA: −0.8-44.2).

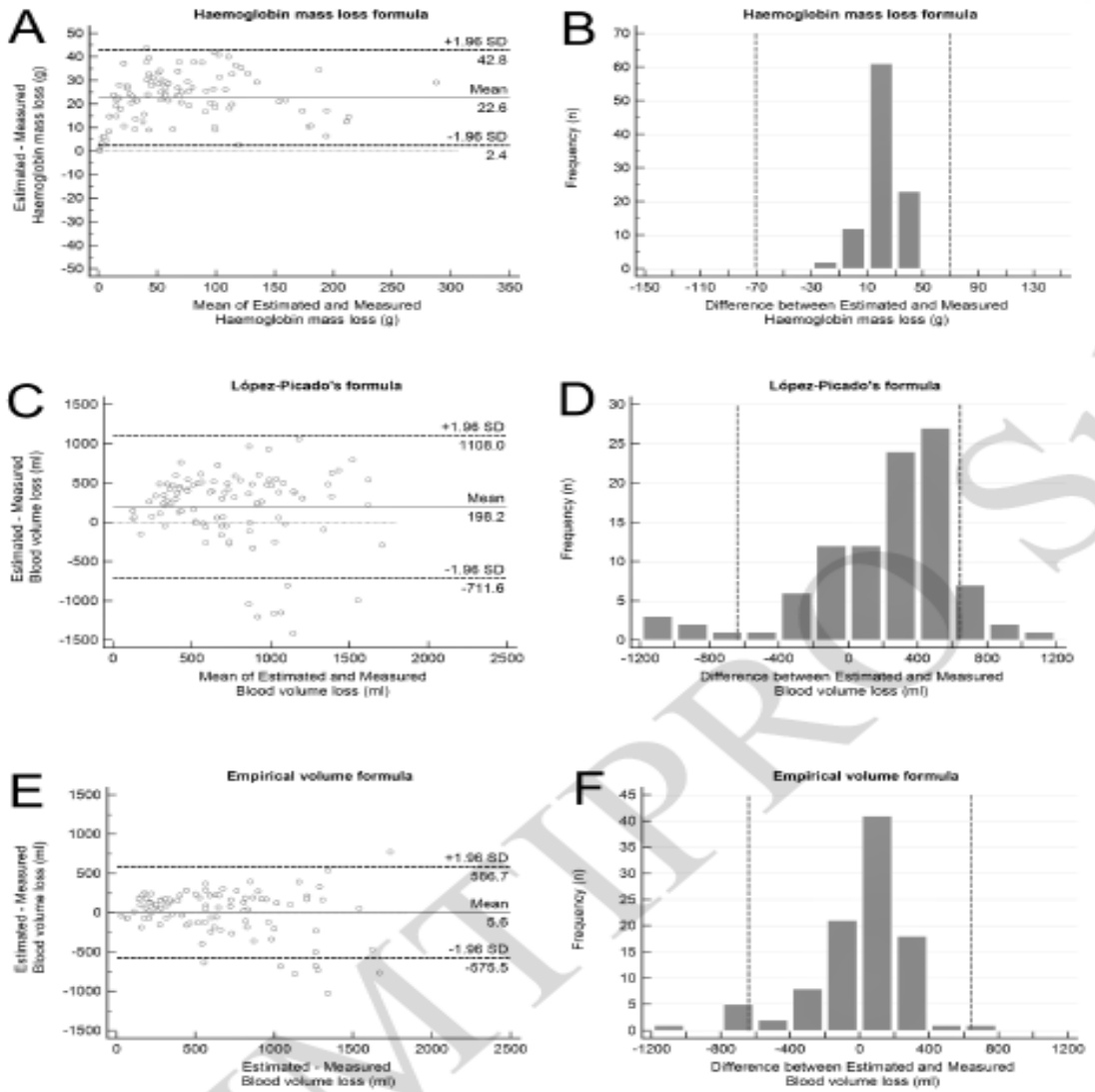

Figure 2 - Bland-Altman plots (left) and histograms of differences between estimated and measured blood loss values (right).

In the Bland-Altman plot, bias is represented by a solid grey line, 95% limits of agreement are indicated by dashed black lines, and the line of equality is indicated by a dashed grey line. In the histograms of differences between estimated and measured values, the unit conversion (±71 g in 2-B, ±560 mL in (D) and (F) is marked by dashed grey lines, and unit scales were set approximately at 2× the unit conversion (±150 g and ±1,200 mL) to facilitate visual comparison among formulae' estimations. Although significant bias was observed, the haemoglobin mass loss formula met the agreement criterion of ±71 g (A). The López-Picado's formula (C) and the empirical volume formula (E) exceeded the ±560 limits of agreement. Using the unit conversion in the histograms of differences, the haemoglobin mass loss formula (B) obtained more accurate estimations than the blood volume loss formulae (D and F). SD: standard deviation.

Agreement's strength

The results of the CCC analysis are summarised in **Table II**. In comparison to the López-Picado's formula and the empirical volume formula, the haemoglobin mass loss formula achieved better precision, accuracy and agreement strength in estimating blood loss.

Table II - *Comparison of the precision, accuracy and agreement strength achieved by the blood loss formulae**

	Precision (ρ)	Accuracy (C_b)	CCC index (ρ_c)
Haemoglobin mass loss formula	0.981 (0.972-0.987)	0.926 (0.902-0.950)	0.909 (0.877-0.933)
López-Picado's formula	0.501 (0.335-0.637)	0.888 (0.774-0.921)	0.445 (0.289-0.577)
Empirical volume formula	0.815 (0.736-0.872)	0.921 (0.895-0.947)	0.751 (0.668-0.826)

*Concordance correlation coefficient parameters are expressed with 95% confidence interval.

Agreement's consistency

The Bland-Altman plots showing percentage differences for each formula are shown in **Figure 3**. The haemoglobin mass loss formula showed that the differences (in percentage) became progressively narrower as blood loss increased (**Figure 3A**). Therefore, the haemoglobin mass loss formula showed considerable consistency. The López-Picado's formula and the empirical volume formula showed a dispersion width that remained unchanged as blood loss increased (**Figure 3B** and **C**, respectively). Thus, it was considered that there was no substantial consistency in the agreement of these blood volume loss formulae and that the accuracy of the estimation deteriorated at greater magnitudes of blood loss, as seen in the Bland- Altman plots in absolute units (**Figure 2C** and **E**).

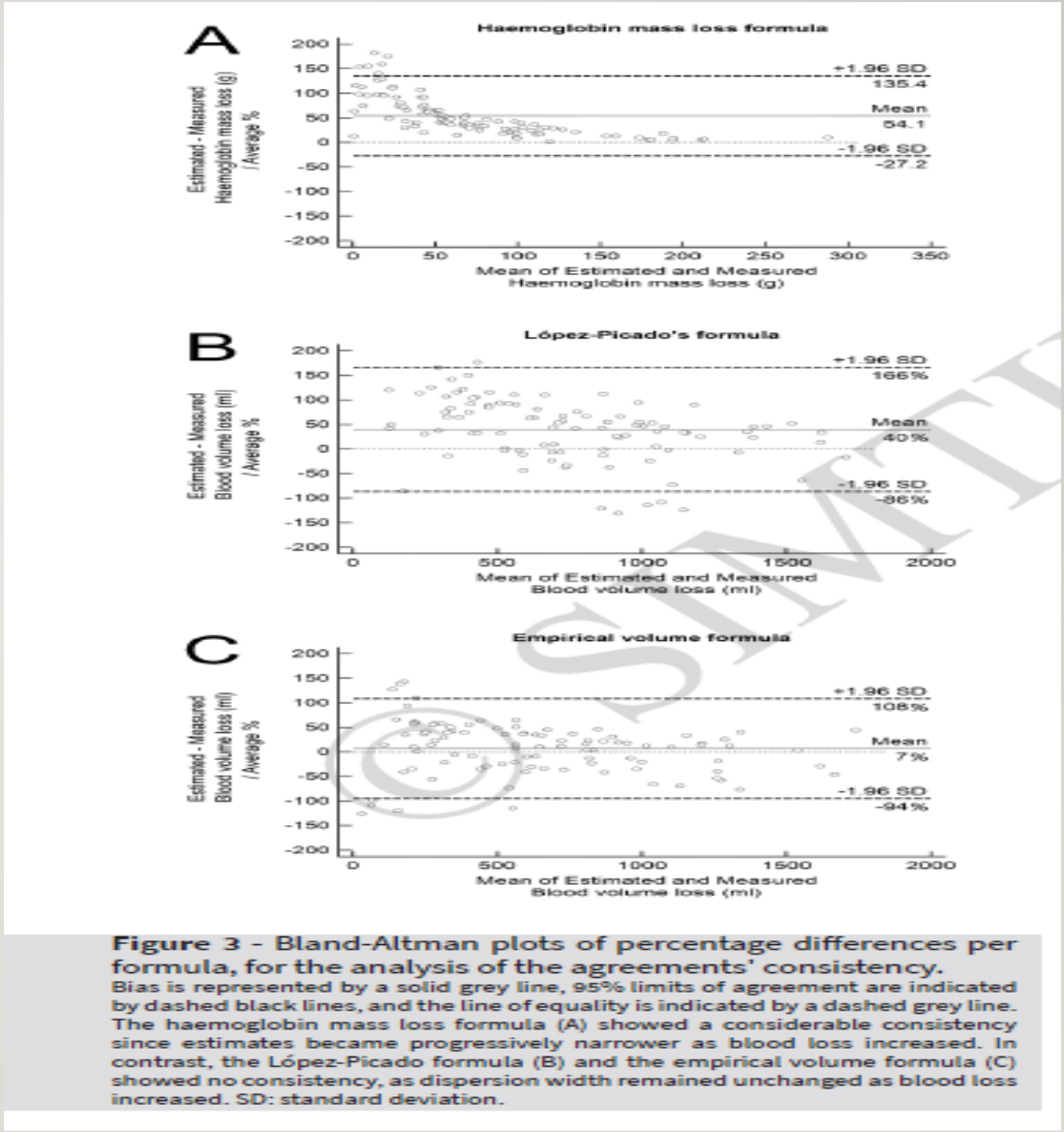

Figure 3 - Bland-Altman plots of percentage differences per formula, for the analysis of the agreements' consistency.
Bias is represented by a solid grey line, 95% limits of agreement are indicated by dashed black lines, and the line of equality is indicated by a dashed grey line. The haemoglobin mass loss formula (A) showed a considerable consistency since estimates became progressively narrower as blood loss increased. In contrast, the López-Picado formula (B) and the empirical volume formula (C) showed no consistency, as dispersion width remained unchanged as blood loss increased. SD: standard deviation.

DISCUSSION

In this study, we evaluated an estimation of the haemoglobin mass loss as an alternative approach to estimate perioperative blood loss for research purposes. A formula was constructed to estimate the haemoglobin mass loss, and then tested and compared to estimation formulae based on blood volume loss, assessing their agreements with their respective measurements of blood loss. The proposed formula yielded estimations that were accurate enough according to the limits of agreement defined in the design of the study, although a moderate overestimation was observed. Using a unit conversion, the haemoglobin mass loss formula approximately estimated 20-338 mL of blood volume loss of an average healthy adult. In comparison to the blood volume loss formulae, the haemoglobin mass loss formula improved the estimation of blood loss, as shown by the different agreement' parameters analysed.

Quantification of perioperative blood loss is a key parameter in clinical studies related to Patient Blood Management. Although transfusion rate and other clinical outcomes may have a greater clinical impact, blood loss, when accurately quantified or estimated, can be a more robust parameter, as it is less inf luenced by confounding factors (e.g., transfusion threshold, haemoglobin mass contained in the RBC units, etc.).

Previous reports have observed significant inaccuracies in the current formulae used to estimate blood loss in volume units. In this study, even when we considered the best possible estimation of blood volume loss, the haemoglobin mass loss formula achieved better agreement with direct measurements of blood loss.

A reasonable explanation for the observed inaccuracies of the blood volume loss formulae is that volume loss might not always be correctly ref lected in the haematological parameters, since blood loss could have variable degrees of dilution (and haemoglobin mass). For the same reason, haemoglobin mass loss could not be converted into volume units, but only be considered in mass units. However, we recognise that, even in highly controlled scenarios, such as the one we intended to accomplish, an accurate measurement of blood volume loss is difficult to achieve. As a result, blood volume loss formulae might never be properly validated in the clinical setting.

We chose to measure haemoglobin mass loss directly from blood loss, as we considered it to be the most reliable measurement. Although RBC labelling or measurements of circulating haemoglobin mass would have been helpful for exploratory analyses, we were interested in the haemoglobin mass loss itself. As acute mobilisations of RBCs between the circulating and non-circulating compartments (e.g., spleen) can occur during bleeding and the intraoperative period[19], measuring circulating haemoglobin mass in the pre-and postoperative periods could ignore the mass lost during those mobilisations and redistributions, and therefore, it may not correctly reveal the actual haemoglobin mass loss.

Despite the agreement achieved by the haemoglobin mass loss formula, a significant bias was observed, resulting in a moderate overestimation of haemoglobin mass loss. Given that it remained nearly constant among different magnitudes of blood loss, possible sources of bias might have been incomplete measurements of blood loss, blood coagulating before reaching the suction system, or blood loss resulting from blood extractions (used for laboratory analysis), although an intrinsic inaccuracy of the formula cannot be ruled out.

This study has several **limitations**. Its main limitation is that patients' total blood volume was estimated but not measured. Although inaccuracies may have occurred, we used this approach as it is a more practical tool for research purposes. Measuring the exact blood volume involves costly proceedings and does not significantly contribute to the estimation of haemoglobin mass loss, since an error of 500 mL would change the estimated haemoglobin mass loss by approximately 8-10%[8].

Euvolaemia was assumed but not measured between pre- and postoperative time points. Although there is evidence to support the euvolaemic condition when patient's haemoglobin concentration reaches the nadir level and fluid balance was not shown to change the accuracy of the estimations, no direct measurements of the volaemic conditions were performed. Bioimpedance measurement devices would be helpful tools for this purpose; while they do not seem to accurately measure the absolute blood volume, they have proven to be accurate to measure individual tendencies and relative changes (such as the return to the preoperative volaemic conditions).

The proposed formula also has the disadvantage of **requiring the patient's lowest haemoglobin concentration**, which necessarily implies measuring haemoglobin concentration at least each day and then make the estimations retrospectively. But again, the proposed formula was designed for research purposes only. On the other hand, studies have observed that haemoglobin concentration stabilises after 2-4 days, and no significant variations occur in the 6-8 days following stabilisation. The use of bioimpedance devices would solve this problem if more precise estimations were needed.

Finally, to test the haemoglobin mass loss formula, we chose an ideal clinical scenario for its evaluation, excluding patients who required transfusion or presented postoperative bleeding. This is important, as several studies that evaluate strategies aimed at reducing perioperative bleeding are performed in scenarios in which blood transfusions and postoperative bleeding are common. But, as discussed above, this study was designed as a first proof of concept. Theoretically, postoperative bleeding would not change the accuracy of the estimations since haemoglobin concentrations would keep falling until the lowest haemoglobin concentration was reached and stabilised. Further studies including these and other types of variables (e.g., type of surgery) would be necessary before it could be applied. The addition of the transfused haemoglobin mass (contained in RBC units) into the equation could be considered in future studies.

CONCLUSIONS

The estimation of haemoglobin mass loss showed a significantly better estimation of perioperative blood loss than blood volume loss formulae. Further validation studies are needed to establish its reliability before its application, especially studies involving postoperative bleeding and blood transfusions

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AUTHORSHIP CONTRIBUTIONS

SJ contributed to the design of the study, statistical analyses, and manuscript preparation. MM-M contributed to patient recruitment, execution of the patients' measurements, and manuscript preparation. PLG contributed to the statistical analyses and manuscript preparation. DC contributed to patient recruitment, execution of the patients' measurements, and manuscript preparation. RN-R contributed to the design of the study and manuscript preparation. AB contributed to the design of the study and manuscript preparation.

The Authors declare no conflict of interest.

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Thanks For Your Attention