

# Targeted Bleeding Management Guided by Non-Invasive Haemoglobin Measurement in Surgical Patients

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## ABSTRACT

**Objective:** To assess blood transfusion decisions in blood losses using a continuous total haemoglobin (SpHb) and non-invasive haemoglobin (Hb) device.

**Study Design:** Double-blinded randomised controlled trial.

**Place and Duration of Study:** Marmara University Hospital, Istanbul, Turkey, from March 2018 to December 2019.

**Methodology:** One hundred and twenty adult patients scheduled for elective major surgery and expected to experience a blood loss greater than 20% of their total blood volume were divided into two groups. These groups were compared for bleeding management with conventional blood gas sampling (Group Hb, the control group) according to Hb monitoring *versus* SpHb measurement (Group SpHb, the study group).

**Results:** In the postoperative measurement, there were fewer red blood cells (RBC) in the SpHb group than in the Hb group ( $p=0.020$ ). There was a greater change in the amount of RBC from the perioperative to the postoperative period in the SpHb group compared to the Hb group ( $p<0.001$ ). Postoperative Hb levels of patients in the intensive care unit (ICU) were higher in the SpHb group than in the Hb group ( $p<0.05$ ).

**Conclusion:** SpHb can provide effective patient blood management in cases of major surgery. It does not cause a delay in the decision of blood transfusion during surgery.

**Key Words:** *Haemorrhage, Anaemia, Blood transfusion, General surgery.*

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## INTRODUCTION

Blood transfusion is a necessary treatment to optimise the continuity of microcirculation and the advantages of transfusion must be balanced with the risks it has. Increased risk for bleeding in major surgeries paves the way for the occurrence of microcirculatory perfusion failure.<sup>1</sup>

Several methods including calculating estimated blood loss, and monitoring vital signs such as heart rate, blood pressure, urine output, and central venous pressure have been used in intraoperative blood transfusion management. However, the most preferred method is to measure haemoglobin (Hb) levels in blood samples which are collected intermittently in the intraoperative period<sup>2,3</sup> but this method is time-consuming.

Complications resulting from a delayed or unnecessary blood transfusion are common in clinical practice.<sup>4-6</sup> The association of blood transfusion with increased mortality and morbidity has led to the emergence of the need for devices to perform continuous Hb measurements. The most significant advantage of continuous Hb measurement is that, it is able to instantly detect sudden decrease in Hb levels and maintains hemodynamic stability by providing Hb concentration within normal ranges. Non-invasive Hb monitoring, which is used to optimise Hb levels in order to increase oxygen delivery to intraoperative tissues, can shed light on the state of microcirculation. Pleth variability index (PVI) (Masimo, Irvine, CA) is a new non-invasive technique based on Perfusion Index (PI) measurements during respiratory cycles, which is a reliable parameter in fluid response.

Pulse Co-oximetry uses pulse oximetry that continuously and non-invasively measures the non-invasive Hb concentration (total haemoglobin, SpHb) using light-absorbing diode sensors of different wavelengths. The method used to measure Hb with Pulse Co-oximetry is similar to the one used while measuring oxyhaemoglobin with conventional pulse oximetry. Continuous monitoring of a patient's clinical state with SpHb monitoring allows transfusion to be performed quickly and accurately. However, the trend and calibration should be evaluated to increase the accuracy of SpHb monitoring, and recommended steps should be followed.

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It was hypothesised that bleeding management with continuous SpHb monitoring during major surgery can reduce transfusion rates and provide more effective hemodynamic control compared to bleeding management which is based on Hb measurement with intermittent blood sampling. The primary purpose of this prospective and experimental study was to determine the effects of SpHb monitoring on intraoperative blood transfusion in patients undergoing major surgery. The secondary aim was to reveal the hemodynamic and metabolic changes taking place intraoperatively.

## METHODOLOGY

This study was a prospective, double-blind, and randomised controlled trial and was approved by Marmara University School of Medicine Ethics Committee with decision number 09.2018.134 and registered in the Clinical Trials Registry (ACTRN1269000129189). To detect a 20% reduction in the amount of blood and blood products given to patients during the intraoperative period, the margin of error was 5%, and the power of the study was considered 90% with a standard effect size of 0.63, and 53 subjects (n=53) per group were considered sufficient.

From March 2018 to December 2019, 120 patients aged between 18 to 85 years with an ASA score of I-III who were scheduled for major surgical operations with an expected blood loss greater than 20% of blood volume were included in the study. Patients who underwent emergency surgery; who had a known severe cardiopulmonary disease such as heart failure or severe chronic obstructive lung disease, abnormal coagulation function prior to surgery, uncontrolled systemic infections, hepatic or renal failure, pregnancy or lactation, who had surgical complications during the operation and whose duration of operation exceeded 10 hours, and those who refused to sign the informed consent form, were excluded from the study.

The patients were divided into two groups of 60 patients with the closed envelope method: Group Hb which underwent Hb monitoring with conventional intermittent blood gas sampling, and Group SpHb which underwent SpHb measurement. Written informed consent were obtained from all the patients.

Electrocardiogram (ECG), peripheral oxygen saturation (SpO<sub>2</sub>), and non-invasive blood pressure monitoring were performed for all patients. Radial artery cannulation was performed with a 20 G cannula and blood gas samples were analysed with a Radiometer, ABL800Flex (Radiometer, Copenhagen, Denmark) device. Routine anaesthesia induction was performed with propofol, rocuronium bromide, and fentanyl in a 50–50% oxygen-air mixture. After intubation, 2% sevoflurane was used for the maintenance of anaesthesia. In the case of intravenous fluid maintenance, the balanced electrolyte solution was adjusted according to the type of surgery, and additional boluses were administered with a colloid solution. When patients developed hypotension despite adequate fluid replacement, the intravenous infusion was initiated with norepinephrine 0.02-0.2 mcg/kg/min a vasopressor agent.

Arterial blood gas analysis was routinely performed in both groups at the beginning, 2<sup>nd</sup> hour, 4<sup>th</sup> hour, and end of the operation. The Hb measurements obtained were recorded in the SpHb group. In case of a sudden decrease in the Hb level, a blood gas analysis was performed. Transfusion management was performed according to blood gas analysis in the Hb group and according to the SpHb monitoring in the SpHb group. Blood transfusion was carried out when the Hb levels fell below 9 g/dL as per the recommendations of the European Society of Anaesthesia on intraoperative bleeding management. Demographics, including age, gender, body weight, and ASA status of the patients were recorded. Mean arterial pressure (MAP), heart rate (HR), SpO<sub>2</sub>, end-tidal carbon dioxide (EtCO<sub>2</sub>), central venous pressure (CVP), PVI in the SpHb group, and blood gas values were recorded every two hours. Furthermore, Hb, haematocrit (Htc), thrombocyte count, creatinine, and international normalised ratio (INR) were recorded at the beginning of the operation.

Intraoperative blood transfusion, crystalloid, and colloid administered, type of surgery, operation duration, duration of anaesthesia, urine output, and need for vasopressor were recorded at the end of the operation. Continuous SpHb was monitored with the adult 25 L Hb probe (Massimo, Irvine, CA) connected to the Radical 7 Pulse CO-oximeter device in the SpHb group. PVI measurement and perfusion index (PI) were also recorded in this group.

All intraoperative complications were recorded. Postoperative need for intensive care and the amount of transfusion given, need for vasopressor in the intensive care unit, the lowest Hb in the intensive care unit, duration of hospital stay; severe monthly arrhythmia in the 1<sup>st</sup> month, pulmonary embolism, myocardial infarction (MI), and cerebrovascular accident (CVA) were investigated and followed up.

The R version 2.15.3 program (R Core Team, 2013) was used for statistical analysis. The Shapiro-Wilk test, which tests the assumption of normality, was used to determine whether the quantitative data were suitable for normal distribution. While the mean and standard deviation (SD) were calculated for the normally distributed quantitative variables, the median and interquartile range (IQR) were calculated for the non-normally distributed quantitative variables. Frequencies and percentages were calculated in the evaluation of qualitative variables. The independent samples t-test was used for the evaluation of normally distributed variables between groups. The Mann-Whitney U test was used for the evaluation of non-normally distributed variables between groups. Repeated measures analysis of variance and Bonferroni-corrected pairwise comparisons were used for intragroup evaluations of variables showing normal distribution with more than two repetitions. The Pearson's chi-square test was used for the comparison of qualitative variables. The Spearman correlation analysis was used to determine the level of correlation between non-normally distributed quantitative variables. Linear regression analysis was used to determine the factors affecting ICU, hospital stay, and the use of

a vasopressor agent. Logistic regression analysis was used to determine the factors affecting the complications and mortality within the 1<sup>st</sup> postoperative month. The  $p < 0.05$  was considered statistically significant.

## RESULTS

From March 2018 to December 2019, 120 patients between 18 to 85 years of age with an ASA score of I-III, who were scheduled for major surgical operations with an expected blood loss greater than 20% of blood volume, were included in the study. The duration of anaesthesia varied between subjects from 4 to 8 hours, and the mean duration of anaesthesia was  $5.94 \pm 0.94$  hours. The surgical intervention duration varied between 3.5 and 7.5 hours, and the average time was  $5.12 \pm 0.80$  hours. No significant difference was found between the groups regarding demographic data (Table I). No significant difference was found between the groups in MAP, heart rate, and arterial blood gas analysis.

In the SpHb group, the observed change in PVI, SpHb, and Pl values over time was found to be significant ( $p = 0.004$ ,  $p < 0.001$ , and  $p < 0.001$ , respectively; Table II). As a result of the Bonferroni corrected pairwise comparisons, the initial values were higher than the values obtained in the last measurement ( $p = 0.004$ ,  $p < 0.001$ , and  $p < 0.001$ , respectively; Table II).

The groups did not show significant difference with regards to Hb, Plt, INR, and creatinine in postoperative measurements; the amounts of red blood cell (RBC), cryoprecipitate, fresh frozen plasma (FFP), and Plt suspension used intraoperatively and postoperatively. The postoperative RBC transfusion rate was lower in the SpHb group than in the Hb group ( $p = 0.020$ ). Postoperative Hb levels of ICU patients were higher in the SpHb group compared to the Hb group ( $p = 0.033$ ). No significant difference was found between the groups in terms of the duration of ICU and hospital stays, the use of vasopressors, preoperative complications, postoperative complications within the 1<sup>st</sup> month, and mortality rate (Table III).

A positive correlation was found between the intraoperative, postoperative, total transfusion rates, and the postoperative ICU stay ( $r = 0.514$ ,  $p < 0.001$ ;  $r = 0.368$ ,  $p < 0.001$  and  $r = 0.551$ ,  $p < 0.001$ , respectively). There was no relationship between intraoperative transfusion and hospital stay. A positive correlation was also found between postoperative, total transfusions, hospital stay, and postoperative vasopressor usage ( $r = 0.317$ ,  $p < 0.001$ ;  $r = 0.334$ ,  $p < 0.001$ ;  $r = 0.450$ ,  $p < 0.001$  and  $r = 0.497$ ,  $p < 0.001$ , respectively).

The amounts of intraoperative, postoperative, and total transfusions performed for the subjects with complications in the first postoperative month were higher than the amounts given to the subjects without complications ( $p = 0.032$ ,  $p < 0.001$ , and  $p = 0.001$ , respectively). The number of intraoperative, postoperative, and total transfusions performed for the subjects with mortality was found to be higher compared to the cases with no mortality ( $p < 0.001$ ).

Multivariable evaluations indicated that cranial surgery, postoperative transfusion, pH, and SpO<sub>2</sub> had a significant effect on ICU stay ( $p < 0.001$ ,  $p < 0.001$ ,  $p = 0.002$ ,  $p = 0.017$ , respectively). Postoperative EtCO<sub>2</sub> and SpO<sub>2</sub> had a significant effect on hospital stay ( $p = 0.037$  and  $p = 0.013$ , respectively). Cranial surgery, postoperative transfusion, and pH had a significant effect on vasopressor use ( $p = 0.003$ ,  $p < 0.001$ , and  $p = 0.015$ , respectively).

Postoperative transfusion, postoperative CVP, lactate, Hb, and Htc had a significant effect on the incidence of complications in the first postoperative month ( $p = 0.008$ ,  $p = 0.025$ ,  $p = 0.021$ ,  $p = 0.013$ , and  $p = 0.020$ , respectively). Intraoperative and postoperative transfusion had a significant effect on mortality ( $p = 0.001$  and  $p = 0.011$ , respectively).

## DISCUSSION

In this study, the effectiveness of blood management was investigated through non-invasive Hb monitoring and its effect on morbidity and mortality in patients expected to lose more than 20% of their total blood volume during elective major surgery. Applying this method can contribute to ensuring less postoperative blood transfusion, shorter stay in the ICU, as well as decreased mortality.

Major surgeries are predicted to last more than two hours and are related to a blood loss of more than 20% of the total blood volume.<sup>7</sup> Major surgeries, duration of surgery, and body mass have been reported to be factors associated with intraoperative blood loss.<sup>8</sup> Delays in blood transfusion may result in impaired tissue perfusion. However, unnecessary blood transfusion should be always kept in mind due to potential reactions and complications related to transfusion. Besides increased cost, unnecessary blood transfusions are also associated with an increase in the duration of hospital stay, the incidence of pneumonia, and mortality.<sup>9-11</sup>

Monitoring of the patient's vital signs and tissue oxygenation indicators is necessary, measurement of Hb concentration in the blood is the method that makes the greatest contribution to the decision whether to perform transfusion or not.<sup>12</sup> This method's applicability depends on intermittent blood sampling, therefore, it is far from offering rapid diagnosis in cases of acute blood loss.<sup>13</sup>

In this study, no significant difference was found between the SpHb group and the Hb group regarding intraoperative RBC transfusion. However, the postoperative RBC transfusion rate was lower in the SpHb group than in the Hb group. RBC transfusions during the surgery were performed at a sufficient amount and at the appropriate time. Postoperative blood transfusion increases mortality. For this reason, in the SpHb group, through the early diagnosis of anaemia during surgery, blood transfusion under general anaesthesia was performed safely before the end of the operation, and in this way, the need for postoperative RBC was reduced. This has also been found to be associated with reduced postoperative mortality.

**Table I: Demographic data.**

	SpHb Mean±sd	Hb Mean±sd	T	<sup>a</sup> p
Age (years)	53.65±14.63	53.97±15.63	-0.115	0.909
BMI (kg/m <sup>2</sup> )	27.63±4.89	27.60±4.66	0.038	0.970
Anaesthesia duration (h)	5.95±0.90	5.94±0.99	0.048	0.962
Operation duration (h)	5.13±0.72	5.1±0.88	0.170	0.865
	n (%)	n (%)	$\chi^2$	p
Gender				
Female	28 (46.7)	23 (38.3)	0.853	<sup>o</sup> 0.356
Male	32 (53.3)	37 (61.7)		
ASA status				
1	11 (18.3)	8 (13.3)	0.575	<sup>o</sup> 0.750
2	43 (71.7)	46 (76.7)		
3	6 (10)	6 (10)		
Surgical intervention type				
Cranial surgery	5 (8.3)	4 (6.9)	10.248	0.069
Major abdominal surgery	15 (25)	10 (17.2)		
Major orthopaedic surgery	15 (25)	14 (24.1)		
Major thoracic surgery	8 (13.3)	7 (12.1)		
Major urological surgery	2 (3.3)	13 (22.4)		
Vertebra surgery	15 (25)	10 (17.2)		

<sup>a</sup>Independent samples t-test, <sup>o</sup>Pearson Chi-square test.

**Table II: Comparison of PVI and SpHb values.**

PVI	SpHb Mean±sd	
Baseline	16.84±9.02	
(2 h)	14.36±7.2	
(4 h)	13.73±7.94	
Last	12.38±5.95	
F; <sup>b</sup> p	F=4.685; p=0.004**	
Change	Mean±sd	<sup>c</sup> p
2 <sup>nd</sup> h - Baseline	-2.48±10.13	0.190
4 <sup>th</sup> h - Baseline	-3.11±12.09	0.154
Last-Baseline	-4.46±10.29	0.004**
4 <sup>th</sup> h - 2 <sup>nd</sup> h	-0.63±8.46	0.500
Last - 2 <sup>nd</sup> h	-1.98±7.6	0.144
Last - 4 <sup>th</sup> h	-1.35±7.13	0.443
SpHb	SpHb Mean±sd	
Baseline	11.91±1.56	
2 <sup>nd</sup> h	10.84±1.81	
4 <sup>th</sup> h	10.14±1.69	
End	10.01±1.7	
F; <sup>b</sup> p	F=43.629; p<0.001**	
Change	Mean±sd	<sup>c</sup> p
2 <sup>nd</sup> h - Baseline	-1.07±1.16	<0.001**
4 <sup>th</sup> h - Baseline	-1.77±1.51	<0.001**
End - Baseline	-1.89±1.6	<0.001**
4 <sup>th</sup> h - 2 <sup>nd</sup> h	-0.7±1.5	0.002**
End - 2 <sup>nd</sup> h	-0.83±1.58	<0.001**
End - 4 <sup>th</sup> h	-0.12±1.21	0.500

<sup>a</sup>Analysis of variance in repeated measures, <sup>b</sup>Bonferroni corrected pairwise comparisons, \*\*p<0.01.

Murphy *et al.* showed in their study that RBC transfusion in cardiac surgery patients is associated with early and late mortality with ischemic postoperative morbidity.<sup>14</sup> In another study, a dose-dependent association was demonstrated between RBC transfusion and increased morbidity and mortality in patients undergoing elective open abdominal aortic aneurysm repair.<sup>15</sup>

Ehrenfeld *et al.* found that the number of patients who underwent intraoperative blood transfusion was higher in the group in which bleeding was managed with a non-invasive haemoglobin probe compared to the group that received standard care in orthopaedic surgeries.<sup>16</sup> Perioperative blood transfusion was higher in the standard care group compared to the non-invasive Hb group. Intraoperative Hb levels were found to be similar between the SpHb group and standard care groups. In our study, no significant difference was found between the SpHb and Hb groups in terms of perioperative RBC use. However, postoperative RBC use was higher in the Hb group. Intraoperative haemoglobin levels were similar in both groups. The Hb levels of the patients in the Hb group were lower in the patients who were followed up in the postoperative intensive care unit. The authors believed that it was due to inadequate perioperative blood management and the inability to provide effective transfusion treatment.

Awada *et al.* found that blood transfusion in the SpHb group was lower compared to the Hb group considering blood transfusions for neurosurgery patients.<sup>17</sup> The results by Awada *et al.* indicated that a greater number of blood transfusions were performed in the Hb group.

Nevertheless, there was no difference between SpHb and Hb groups in terms of blood transfusions performed in the present study. This may attribute to the great variety of cases. However, the amount of blood given in the SpHb group was lower. This finding may suggest that one can reduce postoperative transfusion by optimising the use of blood products in the perioperative period with the use of a non-invasive haemoglobin device.

Tang *et al.* conducted a study on patients who underwent spinal surgery and compared Hb measurements by non-invasive Pulse Co-oximetry with standard care arterial blood gas measurement.<sup>18</sup>

**Table III: Comparison of intraoperative and postoperative data.**

	SpHb Mean±SD (median±IQR)	Hb Mean±SD (median±IQR)	t	P
Preoperative Creatinine	0.77±0.22	0.86±0.52	-1.259	<sup>a</sup> 0.212
Intraoperative haemorrhage	1900.5±1114.53	1940.83±1068.8	-0.202	<sup>a</sup> 0.840
Intraoperative crystalloid	3625.83±1957.98	3570±2036.81	0.153	<sup>a</sup> 0.879
Intraoperative colloid	1020±449.31	989.17±505.82	0.353	<sup>a</sup> 0.725
Intraoperative urine	800±659.92	728.83±587.38	0.624	<sup>a</sup> 0.534
Postoperative fibrinogen	317.2±143.4	235.7±141.92	1.277	<sup>a</sup> 0.218
Postoperative drainage	604.67±922.82	701.33±792.75	-0.615	<sup>a</sup> 0.539
Postoperative 24 <sup>th</sup> h lactate	2.54±3.99	1.68±1.49	1.576	<sup>a</sup> 0.119
Postoperative 24 <sup>th</sup> h creatinine	0.88±0.67	0.86±0.49	0.138	<sup>a</sup> 0.890
Postoperative Hb (total)	8.75±1.28	8.64±1.53	-0.302	<sup>a</sup> 0.763
Postoperative Hb (ICU)	8.41±1.08	7.75±1.19	2.181	<sup>a</sup> 0.033*
Postoperative Hb (ward)	9.09±1.40	9.37±1.39	-0.971	<sup>a</sup> 0.332
Postoperative vasopressor (day)	(0±1)	(0±1)	-0.196	<sup>c</sup> 0.844
	<b>n (%)</b>	<b>n (%)</b>	<b>χ<sup>2</sup></b>	<b>P</b>
Intraoperative vasopressor	21 (35)	28 (46.7)	1.690	<sup>a</sup> 0.194
Intraoperative complication	2 (3.3)	0 (0)	2.034	<sup>b</sup> 0.496
Postoperative complication in the 1 <sup>st</sup> month	10 (16.7)	14 (23.3)	0.833	<sup>a</sup> 0.494
Mortality (exitus)	4 (6.7)	6 (10)	0.436	<sup>a</sup> 0.509

<sup>a</sup>Independent groups t-test, <sup>b</sup>Mann-Whitney U test, <sup>c</sup>Pearson's chi square test, <sup>d</sup>Fisher's exact test.

Since postoperative vasopressor use did not show normal distribution, the Mann-Whitney U test was applied. The results were expressed as median±IQR, and no significant difference was observed.

Tang *et al.* aimed to measure intermittent blood haemoglobin by performing arterial intervention in all patients, and invasive Hb measurement was performed using Co-oximetry in both groups. When the two groups were compared, blood samples were taken with a 1 g/dl decrease in SpHb, so a higher number of blood samples was taken from the other group. Changing values were SpHb>1 g/dl and Co-oximeter>1 g/dl, accuracy correlation was 93% in SpHb, while it was 50% in standard care. Thus, using a non-invasive Hb device can increase the possibility to detect anaemia. The non-invasive Hb device provides blood sampling due to its trend in patients with haemorrhage and prevents delays in making decisions for a blood transfusion by detecting anaemia early. Haemodilution, sudden blood loss, and rapid transfusion affect the accuracy of the non-invasive Hb device.<sup>19</sup> In the present study, no difference was found in intraoperative blood transfusion and anaemia. In some studies, SpHb monitoring has been shown to prevent unnecessary blood transfusion.<sup>20,21</sup> Previously, different techniques including the use of tranexamic acid were introduced to reduce the intraoperative transfusion rate. However, topical tranexamic acid may lead to complications.<sup>22,23</sup>

Kamal *et al.* compared perioperative RBC transfusions between the SpHb group and the control group in major abdominal cancer surgery.<sup>24</sup> RBC transfusion rate was found to be lower in the SpHb group than in the control group. Post-transfusion Hb was found to be lower in the SpHb group. A blood transfusion decision was made earlier in the SpHb group. In this study, on the other hand, the authors found intraoperative RBC transfusion to be similar in both groups which may be attributed to the sample size. The reason for the less blood transfusion in the postoperative period in the SpHb group may be that the intraoperative need for blood transfusion was met when necessary.

The accuracy of the comparison of Hb measurement of Radical-7® Pulse Co-oximeter and Pronto-7 device with Hb measurements by invasive methods has been demonstrated in many studies.<sup>25</sup> The non-invasive Hb device can warn the clinician in cases where blood transfusion is required and contribute to patient blood management by preventing unnecessary blood transfusion through its continuous Hb trend. The device may not replace conventional methods but may be used as an auxiliary monitoring technique to accompany them and enable to recognise patient-related variables early, improving patients' postoperative outcomes. However, studies with larger patient groups are needed.

Radical-7® Pulse Co-oximeter was also used for the assessment of volume and perfusion status. PI and PVI were continuously monitored. In this way, the clinicians became aware of any possible discordance between the Hb level and the volume. This method allows evaluating the fluid response intraoperatively. The reliability and accuracy were improved in different perfusion and volume statuses. Similarly, in this study, in the SpHb group, the change in PVI, SpHb, and PI values over time was found to be significant. Studying with a larger sample group might have increased the reliability of the study.

## CONCLUSION

SpHb measurement in major surgical cases can accompany conventional Hb measurement methods, allowing effective patient blood management practice. It prevents unnecessary blood transfusions by ensuring that the decision of blood transfusion is made at the appropriate time during the surgical intervention and that the appropriate amount of blood and blood products are given to patients. Since, this may

decrease mortality and morbidity by reducing postoperative blood transfusion, the use of such an advanced monitoring method in major surgeries may increase patient safety.

#### ETHICAL APPROVAL:

The study was approved by Marmara University School of Medicine Ethics Committee with decision No. 09.2018.134 and registered in the Clinical Trials Registry (ACTRN 1269000129189).

#### PATIENTS' CONSENT:

Written informed consents were obtained from all the patients.

#### COMPETING INTEREST:

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#### AUTHORS' CONTRIBUTIONS:

SA: Writing-original draft preparation, conceptualisation, methodology.

SUZ: Data curation, visualisation, writing reviewing and editing.

GC: Corresponding author, visualisation, writing - reviewing and editing.

TU: Data curation, validation, investigation.

ZZA: Data curation, validation, supervision.

AS: Supervision, writing- original draft preparation, software, investigation.

All the authors have approved the final version of the manuscript to be published.

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