Determination of Neurocognitive Changes Using Electroencephalography and the Mini-Mental Test in Coronary Bypass Patients Who Underwent Operation with Pulsatile and Non-Pulsatile Cardiopulmonary Bypass

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ABSTRACT

Introduction: The aim of this study was to compare the neurocognitive effects of pulsatile and non-pulsatile perfusion using electroencephalography and the Mini-Mental State Examination (MMSE) in patients undergoing coronary bypass surgery.

Patients and Methods: We created two groups, each containing 11 consecutive randomized patients, who were candidates for coronary bypass surgery. Pulsatile cardiopulmonary bypass (CPB) was conducted in Group I, and non-pulsatile CPB was conducted in Group II. An electroencephalogram (EEG) was performed preoperatively, intraoperatively, 15 minutes after placement of the cross-clamp, and seven days postoperatively. The MMSE was performed preoperatively and on postoperative day seven.

Results: In both groups, pathological EEG waves were detected intraoperatively. However, no statistically significant difference was found between the groups (p>0.05). Urine output was significantly higher at postoperative day 1 in Group I. Overall urine drainage was higher in Group I, but the difference was not statistically significant. Diabetes mellitus, hypercholesterolemia, hypertension, cross-clamp, and perfusion time did not have a significant impact on the formation of pathological EEG waves. The difference between pre- and postoperative MMSE scores was not statistically significant (p>0.05).

Conclusion: No remarkable superiority was shown between CPB pulsatile and non-pulsatile perfusion in our study. Improving physiology of perfusion techniques during CPB and performing further studies on patients at high risk for organ perfusion would suggest more comprehensive perfusion flow strategies.

Key Words: Electroencephalogram; mini-mental state examination; neurocognitive change; non-pulsatile bypass; pulsatile bypass

Pulsatil ve Nonpulsatil Kardiyopulmoner Baypas ile Koroner Baypas Cerrahisi Uygulanan Hastalarda Nörokognitif Değişikliklerin Elektroensefalografi ve Minimenter Test Kullanılarak Değerlendirilmesi

ÖZET

Giriş: Bu çalışmanın amacı; koroner baypas cerrahisi uygulanan hastalarda pulsatil ve nonpulsatil perfüzyonun nörokognitif etkilerini elektroensefalografi (EEG) ve minimenter test kullanılarak karşılaştırmaktır.

Hastalar ve Yöntem: Arıkştırmayla koroner baypas adayı 11’er hastalık iki grup oluşturuldu. Grup I’de kardiyopulmoner baypas (KPB) pulsatil, grup II’de nonpulsatil olarak yürütüldü. Hastalara ameliyat öncesi, cross klamp konduktan 15 dakika sonra ve ameliyat sonrası yedinci günlerde EEG ile birlikte ameliyat öncesi ve sonrası yedinci günlerde minimenter test çalışıldı.

Bulgular: Her iki grupta da ameliyat sonrası patolojik EEG dalgaları tespit edilmiş ancak aralarında fark bulunamamıştır (p>0,05). Grup I’de ameliyat sonrası birinci gününde idrar çiğliği anlamlı yüksek tespit edilmiştir. Toplam drenaj grup I’de yüksek olması rağmen anlamlı bulunmamıştır. Diabetes mellitus, hiperkolesterolemi, hipertansiyon, kros klamp ve perfüzyon süresinin patolojik EEG gelişmesine anlamlı etkisi saptanamamıştır. Ameliyat öncesi ve sonrası minimenter test skorları istatistiksel olarak anlamlı bulunmamıştır (p>0,05).

Sonuç: Çalışmamızda KPB pulsatil ve nonpulsatil perfüzyonun birbirine belirgin bir üstünülüğü göstermedi. KPB sonrasında perfüzyon yöntemlerinin daha fizyolojik hale getirilmesi, organ perfüzyonu açısından daha yüksek risk grubundaki hastalarda çalışmalara yapılmasını, perfüzyon akım stratejilerini daha iyi ortaya köşmemektedir.

Anahtar Kelimeler: Pulsatil baypas; nonpulsatil baypas; elektroensefalografi; minimenter test; nörokognitif değişiklik

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INTRODUCTION

Normal blood circulation has a pulsatile characteristic depending on systolic and diastolic functions of the heart. It is believed that pulsatile circulation has the most significant impact on microcirculation, and that pulse waves keep the capillary bed open via a direct effect while also mediating normal reflex vasomotor control by stimulating baroreceptors. Normal blood flow is pulsatile, whereas standard cardiopulmonary bypass (CPB) creates non-pulsatile flow and circulation. This circulation type may cause various physiopathological changes in the organism. The essential pathologies are arteriovenous shunting resulting from the flow changes in the microcirculation, venous pooling, and consequent impaired tissue perfusion impairments.

Although improvements in the techniques used for cardiac surgery, anesthesia, and CPB have significantly reduced stroke and mortality rates, the incidence of cognitive decline is still common. Almost 60% of the patients who underwent coronary artery bypass grafting experienced cognitive decline. Cognitive dysfunction after CPB has been explained by 3 essential mechanisms: hypoperfusion, intraoperative cerebral microemboli, and systemic inflammatory response. Because pulsatile CPB provides lower systemic vascular resistance and higher oxygen consumption, it is accepted as being more physiological than non-pulsatile flow. Although pulsatile perfusion is theoretically superior, no consensus has been established among studies on this subject. In this study, we aimed to evaluate the effects of pulsatile and non-pulsatile CPB on the cerebral system by performing electroencephalography (EEG) recordings and neurocognitive tests.

PATIENTS and METHODS

Ethics committee approval was received for this study from the Ethics Committee of the Ankara Training and Research Hospital (Decision Number: XVIII-104212; Decision Date: June 6, 2004).

The present study included 22 consecutive patients who underwent operation under elective conditions for the first time as a result of a diagnosis of coronary artery disease between May 2003 and October 2003. All patients admitted for operation and included in this study were evaluated based on patients’ accounts of neurological disease and bilateral carotid artery Doppler ultrasound for carotid atherosclerotic occlusion. All patients with positive neurological disease and carotid atherosclerotic occlusion (with or without the need for surgery) were excluded from the study. Patients with preoperative renal dysfunction were also excluded from the study. The remaining patients were randomized into 2 groups: pulsatile (Group I) and non-pulsatile (Group II).

EEG recording was performed using digital Nihon Kohden EEG-9100 (version 03-13; Tokyo, Japan). The planned procedures were approved by the Training and Planning Committee of the hospital. The patients were taken into a silent room for preoperative EEG and informed about the procedure. Electrodes were placed on the patients in the appropriate sites using electrode paste. Monopolar recording was performed for 3 minutes in the patients. Subsequently, bipolar EEG recording was performed for 10 minutes with additional stimulations (eyes-opened and eyes-closed tests, inspirium, expirium, and exposure to visual stimuli by photocell). The same procedures were repeated on postoperative day 7. Intraoperative EEG monitors were connected to the intubated patient, and EEG monitoring was performed continuously until the end of the operation. The 3-minute EEG recordings were obtained in different 5 intervals: prior to CPB, 15 minutes after placement of the aortic cross-clamp, after removal of the aortic cross-clamp, and at the end of CPB. In our study, we relied especially on the recordings obtained during CPB and after placement of the aortic cross-clamp. At least 40 seconds of artifact-free recordings were used for the assessments.

All patients were administered 0.07 mg/kg midazolam and 0.1 mg/kg morphine sulfate via the intramuscular route 1 hour before the operation. Invasive arterial pressure monitoring was applied through the radial artery. Anesthesia was induced by intravenous administration of 1.2 mg/kg fentanyl, 4.7 mg/kg pentothal, and 0.20 mg/kg cisatracurium. The central venous catheter was inserted through the right jugular vein. Anesthetic maintenance was performed via repeated administration of 50% O2, 1% isoflurane, 0.02 mg/kg cisatracurium, and 1 µg/kg fentanyl at 30-minute intervals.

Each patient was administered 5 mg/kg heparin sodium intravenously for systemic heparinization. Pump suction was used when the activated coagulation time (ACT) reached 250 seconds and above. Extracorporeal circulation was initiated when the ACT exceeded 480 seconds. The ACT level was tested once every 20-30 minutes. An additional dose of heparin was administered to maintain an ACT level above 400 seconds if needed. By termination of extracorporeal circulation, protamine sulfate was administered intravenously according to the previously prepared dose-response curve. The ACT level was tested 20 minutes later and an additional dose of protamine sulfate was administered if the ACT level was high.

Isothermic blood cardioplegia with high potassium was used to achieve cardioplegia. After CPB was started, an induction dose of 1000 mL of blood was prepared as a separate line through a reservoir and administered via an antegrade induction dose of 10 mL/kg. The maintenance cardioplegic solution was delivered via antegrade maintenance doses of 5 mL/kg at 10 to 15-minute intervals.

Extracorporeal circulation was achieved using a Sarns 7000 Heart–Lung Bypass Machine (3M, St. Paul, MN, USA). A CPB perfusion system consisting of a Medtronic Trillium Affinity NT oxygenator (Minneapolis, MN, USA), a polyvinyl chloride tub-
ing system, and an arterial filter system was used in all patients. The pump flow rate was adjusted to 2.4 l/min/m². The acid-base balance was adjusted using alfa-stat management. The blood glucose level was maintained at approximately 200 mg/dL.

In Group I, pulsatile flow perfusion was initiated after the heartbeat was stopped using an isothermic blood cardioplegic solution. Perfusion was continued by obtaining a pulse wave at a mean of 40-45 mmHg blood pressure in the radial artery by setting the lowest pulse wave value as 25%-30% of the highest pulse wave value, a pulse wave width of 50% and 70 beats per minute (bpm). In Group II, CPB was performed as non-pulsatile with the minimum arterial blood pressure maintained at 45-50 mmHg.

After surgical median sternotomy and aortic unicaval cannulation, the heart was decompressed using a vent inserted into the left atrium. The aortic root and retrograde cannula were used for cardioplegia. The left internal mammary artery and saphenous vein were used for grafting in all cases. The cross-clamp was removed after distal anastomoses. Proximal anastomoses were performed using a side-biting aortic clamp.

The Mini-Mental State Examination (MMSE) was given to the patients on the first preoperative day after admission and again on postoperative day 7. The patients were determined to be educated or uneducated for this assessment, with the patients who were primary school graduates or above considered to be educated. Different forms of the MMSE were used for the 2 groups. The patients were taken to a silent room and could use their audio and visual devices if present. The patients were informed that they would be asked some questions. The questions were repeated up to 3 times when the questions could not be understood or the patient did not attempt to respond. The next question was asked when no response was obtained despite the question being repeated. During the examination, a previously prepared paper was used on which a command, such as “CLOSE YOUR EYES” was written legibly in capital letters on 1 side while 2 interlocking pentagons were drawn to form a 4-sided figure on the reverse side. The patients were asked questions in 5 different categories, including orientation, registration and recall, attention and calculation, memory, and language. There were 10 questions in the orientation category, and 1 point was given for each accurate response. In total, there were 3, 5, 3, and 9 points possible for the registration and recall, attention and calculation, memory, and language categories, respectively. The patients earned a total of 30 points if they responded accurately to all questions.

Statistical Analysis

Non-parametric tests were used in the statistical analysis. The Mann-Whitney U test was used for analysis of the differences between 2 groups. Intragroup differences were evaluated using Wilcoxon signed-ranks test. The significance level was accepted as p > 0.05. The results were expressed as mean, standard deviation, maximum, and minimum values. The determinants for the formation of pathological intraoperative EEG waves were analyzed using linear regression analysis.

RESULTS

Preoperative Results

The groups were similar in terms of age and sex, presence of hypercholesterolemia, diabetes, and hypertension. Preoperative MMSE test scores revealed no difference between the groups. The preoperative EEG recordings in both groups presented the dominance of alpha waves in all patients except 4 with dominance of beta waves. No pathological wave was detected (Table 1).

Intraoperative Results

No statistically significant difference was found between the groups with respect to the number of distal anastomoses, total drainage, hematocrit values at pump, partial pressure of carbon dioxide (pCO₂) at pump, urine output during pump, durations of cross-clamping, and perfusion during pump. In Group II, 3 patients needed inotropics after CPB. However, no significant difference was detected between the groups in terms of need for inotropic support. Pathological theta waves were detected in the intraoperative EEG recordings obtained 15 minutes after the placement of the cross-clamp in both groups. A pathological sharp wave was encountered in 1 patient, but statistically significant difference was encountered between the groups (Table 2). According to regression analysis, age, sex, hypercholesterolemia, diabetes, hypertension, hematocrit values at pump, pCO₂ at pump, duration of cross-clamping, duration of pumping, and preoperative MMSE scores were not found as determinants of pathological EEG waves (Table 2).

Postoperative Results

The results of EEG recordings of the patients on postoperative day 7 were the same as the results of preoperative EEG recordings. No pathological wave was encountered. The postoperative MMSE scores of both groups were similar. There was no difference between discharge durations. Total drainage was higher in Group I, but the difference was not significant. The amount of urine output on postoperative day 1 was statistically significantly higher in Group I patients than Group II (p = 0.006) (Table 3).

None of the patients died in either group. There were no occurrences of renal failure, hemorrhagic diathesis, or similar complications. No early neurological deficit was detected in ei-
Table 1. Preoperative characteristics of patients in the pulsatile and non-pulsatile perfusion groups

<table>
<thead>
<tr>
<th>Preoperative characteristics</th>
<th>Group I (n=11)</th>
<th>Group II (n=11)</th>
<th>Difference between groups, p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>58.45 ± 2.18 (48-70)</td>
<td>55.36 ± 2.84 (40-73)</td>
<td>NS*</td>
</tr>
<tr>
<td>Male/female ratio</td>
<td>9/2</td>
<td>9/2</td>
<td>NS*</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>7/11</td>
<td>3/11</td>
<td>NS*</td>
</tr>
<tr>
<td>Diabetes</td>
<td>2/11</td>
<td>4/11</td>
<td>NS*</td>
</tr>
<tr>
<td>Hypertension</td>
<td>4/11</td>
<td>2/11</td>
<td>NS*</td>
</tr>
<tr>
<td>Preoperative mini-mental test score</td>
<td>25.09 ± 1.48 (13-30)</td>
<td>21.82 ± 3 (3-30)</td>
<td>NS*</td>
</tr>
<tr>
<td>Preoperative pathological EEG waveforms</td>
<td>0</td>
<td>0</td>
<td>NA**</td>
</tr>
</tbody>
</table>

* Non-significant (p > 0.05).
** Statistics are not applicable.
EEG: Electroencephalography, NA: Not applicable, NS: Not significant.

Table 2. Intraoperative characteristics of patients in the pulsatile and non-pulsatile perfusion groups

<table>
<thead>
<tr>
<th>Intraoperative characteristics</th>
<th>Group I (n=11)</th>
<th>Group II (n=11)</th>
<th>Difference between groups, p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distal coronary artery bypass graft anastomoses</td>
<td>2.64 ±0.24 (1-4)</td>
<td>2.27 ± 0.14 (1-4)</td>
<td>NS*</td>
</tr>
<tr>
<td>Hematocrit on cardiopulmonary bypass</td>
<td>24.45 ± 0.81 (20-28)</td>
<td>24.85 ± 1.21 (18.0-29.3)</td>
<td>NS*</td>
</tr>
<tr>
<td>pCO₂ during cardiopulmonary bypass</td>
<td>30.18 ± 1.31(23-35)</td>
<td>24.85 ± 1.21 (26.7-39.0)</td>
<td>NS*</td>
</tr>
<tr>
<td>Cross-clamp time (min)</td>
<td>54.45 ± 5.02 (32-80)</td>
<td>41.70 ± 4.77 (26-71)</td>
<td>NS*</td>
</tr>
<tr>
<td>Cardiopulmonary bypass time (min)</td>
<td>93.54 ± 7.24 (56-130)</td>
<td>73.80 ± 6.74 (50-111)</td>
<td>NS*</td>
</tr>
<tr>
<td>Urine output during CPB (mL)</td>
<td>1140 ± 887 (100 ± 600)</td>
<td>200 ± 40.45 (100-500)</td>
<td>NS*</td>
</tr>
<tr>
<td>Inotrope use</td>
<td>0</td>
<td>3/11</td>
<td>NS*</td>
</tr>
<tr>
<td>Intraoperative pathological EEG waveforms</td>
<td>9/11</td>
<td>6/11**</td>
<td>NS*</td>
</tr>
</tbody>
</table>

* Non-significant (p > 0.05).
** Pathological sharp wave in a patient.
EEG: Electroencephalography, NA: Not applicable, NS: Not significant.

Table 3. Postoperative characteristics of patients in the pulsatile and non-pulsatile perfusion groups

<table>
<thead>
<tr>
<th>Postoperative characteristics</th>
<th>Group I (n=11)</th>
<th>Group II (n=11)</th>
<th>Difference between groups, p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine output on postoperative day 1 (mL)</td>
<td>3680 ± 703 (1900-9700)</td>
<td>2131.82 ± 225.56 (1500-3650)</td>
<td>0.006</td>
</tr>
<tr>
<td>Postoperative pathological EEG waveforms</td>
<td>0</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>Postoperative total blood loss</td>
<td>1236.36 ± 286.66 (300-3600)</td>
<td>780 ± 135.18 (130-1960)</td>
<td>NS</td>
</tr>
<tr>
<td>Mini-mental test score</td>
<td>24.64 ± 1.56 (14-16)</td>
<td>20.91 ± 2.61 (5-29)</td>
<td>NS</td>
</tr>
<tr>
<td>Hospital length of stay</td>
<td>7.78 ± 1.17 (5-17)</td>
<td>7.44 ± 0.89 (14-4)</td>
<td>NS</td>
</tr>
</tbody>
</table>

EEG: Electroencephalography, NA: Not applicable, NS: Not significant.
ther group. Pre- and post-operative MMSE scores were similar in the pulsatile perfusion group. Similarly, no statistically significant difference was found between the pre- and postoperative MMSE scores in the non-pulsatile perfusion group (Table 3).

**DISCUSSION**

This controlled trial was conducted on 2 groups of consecutive and randomized patients who underwent pulsatile and non-pulsatile perfusion in our clinic. As expected, pathological EEG waves were detected in 6 of 11 patients in the non-pulsatile group. However, pathological EEG waves were also encountered in 9 of 11 patients in the pulsatile perfusion group. No statistically significant difference was found between 2 groups.

Although, 2 previous studies on the effects of pulsatile blood flow have shown that there was no difference between pulsatile and non-pulsatile flows in terms of neurological and neuropsychological outcomes, Murkin et al. have demonstrated that postoperative central nervous system dysfunction was more common in patients who underwent coronary bypass(9,10).

In our study, patient groups were not at high risk for cerebral complications in terms of comorbid factors. A high risk group is needed to observe the positive cerebral effects of pulsatile perfusion. On the other hand, the pulsatile pump cannot generate a full physiological pulse waveform. However, it is determined that generation of pulsatile flow by roller pump in the clinical practice caused no change in the brainwave patterns.

In our study, we attempted to keep the mean arterial pressure (MAP) at 50 mmHg to minimize cerebral complications in both groups(11). Mild to moderate hypothermia was achieved. The acid-base balance was adjusted using the alpha-stat approach. Excessive decline in PCO₂ was prevented. Mild hemodilution (hematocrit, 18-29) was achieved. In addition, we aimed to increase the brain and body perfusion by pulsatile perfusion during cross-clamp in Group I. The factors that affect cerebral blood flow during CPB are cerebral metabolic rate of oxygen, pCO₂, hematocrit, and MAP(12-14). Cerebral oxygen distribution delivery may be impaired if MAP declines below 50 mmHg. Novel studies on temperature have suggested that

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**Figure 1.** Pre-, intra-, and post-operative encephalography recordings and pathological changes of a study patient before, during, and after surgery. Alpha waves are dominant in the pre- and post-operative measures, while the pathological theta wave is dominant during surgery.
mild hypothermia (32°C-35°C) during CPB protects the central nervous system until lower temperatures (25°C-30°C) and strict normothermia (36°C-37°C) increases risk for stroke and probable neurocognitive impairment. Use of alpha-stat acid-base management is recommended with respect to neurological outcome during moderate hypothermic CPB. However, pH-stat management is recommended in deep hypothermia (> 20°C). The impact of pCO₂ on outcome is associated with the type of cerebral stress. Alpha-stat management is probably essentially critical in reduction of atheroembolic risk in the adults UNDERGOING operation with moderate hypothermia.

Although pathological EEG waves may emerge beginning with induction of anesthesia by intraoperative EEG during non-pulsatile perfusions, they become more common after placement of the cross-clamp. These pathological waves are not determinants of the permanent brain damage, but emerging pathological waves (usually theta waves) indicate decreased cerebral perfusion. An increase in CBF may be expected after pulsatile perfusion during CPB after placement of the cross-clamp. Advocates of pulsatile CPB have postulated that pulsatile flow primarily increases CBF, cerebral microcirculation, and tissue oxygenation. Furthermore, pulsatile flow prevents abnormal hypothalamic pituitary adrenal function (thyrotropin-releasing hormone and adrenocorticotropic hormone response) seen with non-pulsatile perfusion. This fact shows that cerebral perfusion is protected better by pulsatile flow. However, there is no clinical evidence of better cerebral perfusion and neurological outcomes with pulsatile flow.

EEG may not reflect cerebral blood flow or hypoxia. EEG records only superficial cortical activity. EEG is not expected to detect focal ischemia in the small areas of deep brain regions when most of the neurological damages are originally embolic. In addition, anesthetic agents in the brain basement and hypothermia may change the strength and frequency spectrum of EEG.

MMSE scores showed no significant difference between the groups. Although late-term outcomes were not analyzed in the present study, it is expected that no remarkable neurocognitive deficit remains in the patients, considering that the MMSE is correlated with radiological measurements such as cranial tomography abnormalities, cerebroventricular volumes, and deficits of cerebral perfusion encountered by single-photon emission computerized tomography and that MMSE scores obtained on postoperative day 7 were similar to preoperative scores in our study.

In cardiac surgery, the rate of the patients with multiple risk factors for neurological damage increases as the age of the patient population increases. Hypertension and diabetes are observed in 55% and 25% of the cardiac surgery patients, respectively. Fifty percent or greater carotid stenosis is seen in 15% of cardiac surgery patients, and 13% of those may have experienced transient ischemic attack or previous stroke. These entities may increase stroke risk by 2-fold in cardiac surgery patients. Age, hypertension, and hypercholesterolemia were not found to be determinants of pathological EEG values in our study.

Another study has failed to prove that bypass time caused a decline in CBF. However, experimental modalities have shown that increased blood glucose leads to increased neurological ischemia. In our study, diabetes, duration of cross-clamp, and total perfusion time were not found to be the determinants of formation of pathological EEG waves.

In the present study, total drainage in the pulsatile group was higher, but the difference was not statistically significant (1280 mL vs. 780 mL). One of the disadvantages of pulsatile pump use is the concern that shaped components of the blood may be further damaged. Use of the pulsatile pump may have played a role in the increased total drainage.

Renal failure did not develop in any of the patients. Urine output was found to be significantly higher in Group I than Group II (p= 0.006). This is an estimated outcome, as it is known that pulsatile flow increases renal blood flow and subsequently urine output. Although, no serious renal dysfunction was detected in either group, the renal protective value of the pulsatile flow may be more significant for patients with impaired renal functions at baseline.

At the present time, use of pulsatile perfusion has decreased. There may be some probable reasons for this, such as lack of a satisfactory definition of pulsatile flow, different flow characteristics of different pulsatile pumps, and newly developed membrane gas exchange devices with a higher absorption effect on the pulse waves.

Since the introduction of CBP for use in open heart surgery, many studies and experimental modalities have been developed for pulsatile delivery or physiological delivery of flow pump. This issue has almost become a passion. However, only pulsatile roller pumps are preferred in clinical practice. It is obvious that these pumps cannot imitate physiological flow exactly, especially because hollow fiber membrane oxygenators absorb energy. Further studies are needed to obtain improved physiological pulsatile flow.

In our study, there was no remarkable superiority shown between CPB pulsatile and non-pulsatile perfusion. Improving physiology of perfusion techniques during CPB and performing further studies on high risk patients for organ perfusion would suggest more comprehensive perfusion flow strategies.
**Ethics Committee Approval:** Ethics committee approval was received for this study from the Ethics Committee of the Ankara Training and Research Hospital (Decision Number: XVIII-104212; Decision Date: June 6, 2004).

**Informed Consent:** Written informed consent was obtained from patients who participated in this study.

**Peer-review:** Externally peer-reviewed.

**Author Contributions:** Concept/Design – HE; Analysis/Interpretation – HE; Data Collection – HE; Writing – HE, OVD; Critical Revision – HE; Final Approval – OVD; Statistical Analysis – OVD; Overall Responsibility – HE, OVD

**Conflict of Interest:** The authors have no conflict of interest to declare.

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