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Emergency coronary artery bypass surgery in octogenarians with acute coronary syndrome: off and on pump

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Abstract. – OBJECTIVE: The risk of operation in cardiac surgery increases logarithmically with advanced age. In older individuals, additional comorbidities compel the clinician to deal with postoperative complications. The mortality and morbidity rates of emergency coronary artery bypass surgery (CABG) in the presence of acute coronary syndrome (ACS), or just after it, are higher than those of elective surgeries. In our study, we compared the outcomes of off-pump coronary bypass (OPCAB) and on-pump coronary bypass surgery (ONCAB) in this high-risk subgroup.

PATIENTS AND METHODS: 383 octogenarians who underwent isolated emergency CABG due to ACS were divided into two groups according to the coronary bypass technique. Group 1 (off-pump) median age (IQR) 84 years (min: 80-max: 99, n = 130); Group 2, (on-pump) median age 85 years (min: 80-max: 89, n=253). Preoperative, intraoperative, and postoperative data were collected retrospectively on standard variables. OPCAB and ONCAB outcomes were compared.

RESULTS: ONCAB patients had a significantly longer intensive care unit stay, longer hospital stay, more transfused erythrocyte suspension, more low cardiac output syndrome and acidosis, a higher rate of acute renal failure and a higher rate of stroke than OPCAB patients (respectively; p=0.003; p=0.008; p=0.002; p=0.031; p=0.038, p=0.022, respectively).

CONCLUSIONS: We showed that emergency OPCAB as a revascularization option in elderly patients with acute coronary syndrome is more advantageous in terms of preventing major postoperative complications.

Key Words:
Emergency coronary bypass, Acute coronary syndrome, Octogenarian, Beating heart bypass surgery.

Introduction

The prevalence of atherosclerotic coronary artery disease in the elderly population is high[1]. The patient group was defined as elderly for cardiac surgery; with the increase in expected average age, this definition shifted toward age 80 and older[2-4]. Although advanced age is considered a high preoperative risk factor for elective open-heart surgery, better surgical outcomes have been achieved with modern surgical strategies. Thus, acceptable mortality rates and progressively improving long-term survival outcomes in relation to open heart surgery in elderly patients have been observed[5,6]. It has also been reported that functional outcomes are better after surgery than after medical treatment[7]. Open heart surgery is also commonly performed on elderly individuals, who are considered a high-risk group and have many other problems in addition to heart disease[8]. Recent studies[3,4,9] show near-perfect surgical results and improved postoperative quality of life, especially in elderly patients, after coronary bypass. This result can be explained by recent advances in myocardial protection, surgical techniques, and experience. Elderly patients admitted for open heart surgery may have impaired ventricular function, extensive coronary lesions, impaired respiratory and kidney function, and previous cardiac surgery and may require a longer and more complex or urgent surgical procedure.

Acute coronary syndrome (ACS) resulting from severe atherosclerotic coronary artery disease ranges from severe angina to acute myocardial infarction (AMI) and requires urgent intervention. In patients with unstable angina or

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AMI, primary percutaneous coronary intervention (PCI) is performed as a primary reperfusion therapy, with widespread use in the last decade, to relieve symptoms in emergency conditions[10]. However, there are several conditions and indications for patients with ACS that require immediate coronary artery bypass grafting (CABG). These factors include continuing ischemia, which is unresponsive to optimal medical therapy or successful PCI, unsuccessful PCI, cardiogenic shock, three-vessel disease, or sustained ischemia in the left main coronary artery[11,12]. In-hospital mortality and morbidity rates of CABG surgery are higher than elective surgeries[13]. The operative mortality rates for emergency on-pump coronary bypass (ONCAB) are 1.6-3.2%[12].

CABG procedures have evolved over the past three decades. The off-pump coronary bypass (OPCAB) procedure without the use of cardiopulmonary bypass (CPB), aortic cross-clamping, or cardioplegic arrest has gained significant popularity worldwide due to the reduction in perioperative adverse conditions associated with conventional surgery. CPB can cause increased inflammatory reactions, hemodynamic instability, and neurological dysfunction[14,15]. Myocardial tissue, whose functions are reduced due to ACS, is also exposed to ischemia due to cardioplegic arrest during CBD. OPCAB surgery is relatively advantageous as it does not impose a secondary ischemia burden on the damaged myocardial tissue. As aortic cross-clamping and cardioplegic arrest are not performed in OPCAB, cardiac output and the natural coronary artery flow are preserved[16,17]. The benefits of OPCAB surgery have been demonstrated in patients with comorbidities such as diabetes, renal failure, impaired left ventricular function, or advanced age[17-19]. However, the role of OPCAB surgery in the case of AMI is controversial. Although OPCAB is associated with reduced early mortality[12,20,21], many studies[19,22-24] have defined incomplete revascularization as one of its disadvantages.

This study aimed to compare patients who underwent OPCAB surgery with patients who underwent ONCAB among octogenarians with ACS who required emergency surgery in our clinic and to evaluate the postoperative mortality and morbidity associated with these procedures.

Patients and Methods

A total of 398 patients over the age of 80, who underwent isolated emergency CABG surgery at a tertiary heart center in March 2010 and December 2020, were retrospectively reviewed. 12 patients were excluded from the study due to exclusion criteria and insufficient data. 3 patients were excluded from the study because they died in the preoperative period. Patients who had combined procedures or beating-heart on-pump operations and those who were converted from off-pump to on-pump CABG were excluded. 383 octogenarians who underwent isolated emergency CABG were divided into two groups according to the coronary bypass technique: 130 had OPCAB, and 253 had ONCAB (Figure 1). The emergency status of these patients was based on the latest myocardial revascularization guidelines of the European Society of Cardiology[25]. According to these guidelines, ongoing ischemia despite maximum medical therapy, acute evolving myocardial infarction (ST-elevated or non-ST elevated), and cardiogenic shock with or without support are defined as ACS and need urgent revascularization[25]. The criteria used to select either technique were based on the hemodynamic status of the patient, the anatomy of their coronary vessels, and associated risk factors. Patients with diffuse multivessel disease, poor-quality vessels, or evidence of coronary calcification were considered for CABG. Patients with associated comorbidities, a low ejection fraction (EF), and suitable coronary anatomy (accessible vessels, no evidence of calcification, and good vessel size) were considered candidates for OPCAB. Unstable patients were initially supported with inotropic drugs, intra-aortic balloon pumping, or mechanical ventilation. Further evaluation was made intraoperatively, taking into consideration the size of the heart and the effect of manipulation on hemodynamics. Those with a reasonable heart size who tolerated manipulation underwent OPCAB, otherwise, conventional CABG under CPB was used.

Group 1: OPCAB, median age (IQR) of 84 years (min: 80 - max: 99, n=130); Group 2: ONCAB, median age of 85 years (min: 0 - max: 89, n=253). All patient data were retrospectively entered into an institutional database by the hospital computing system. Emergency CABG was defined as surgery within 12 hours due to anatomical (critical left main stenosis) or unstable symptoms after catheterization. Emergency CABG was defined as surgery within 12 hours due to ongoing ischemia, acute coronary syndromes, hemodynamic instability, and arrhythmias. Stenosis in angiography greater than 50% in the left main coronary artery was defined as significant. Patients

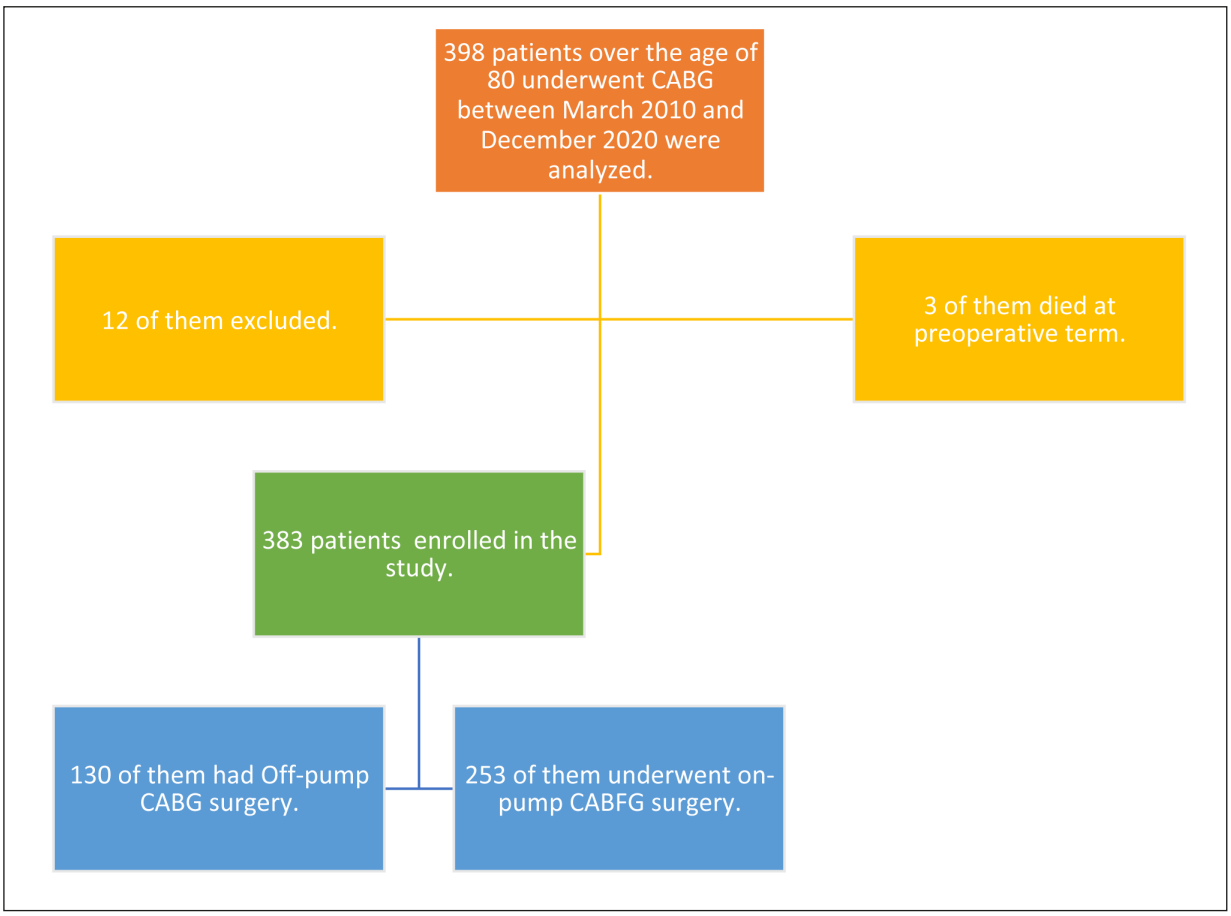

Figure 1. The study flow chart shows the number of patients.

with recurrent angina after catheterization were followed up in the cardiac intensive care unit and referred to emergency surgery. The current analysis is limited to emergency CABG surgery and, consequently, patients with ostial left main coronary lesions, a complication of PCI (e.g., coronary dissection or perforation, acute stent thrombosis), or unstable symptoms in acute coronary syndrome (hypotension, dynamic electrocardiographic changes or arrhythmias). Preoperative, intraoperative, and postoperative data were collected on standard variables. Preoperative risk factors in both groups were evaluated, including concomitant pathologies such as age, diabetes mellitus, chronic obstructive pulmonary disease (COPD), NYHA class grade, smoking, hypertension (HT), hyperlipidemia (HL), previous myocardial infarction (MI), atrial fibrillation (AF) rhythm, previous cerebrovascular disease, carotid artery disease, and preoperative left ventricular ejection fraction values. The use of the left internal mammary artery (LIMA), number of grafts, cardiopulmonary bypass, and cross-clamp times in patients who underwent pump surgery were evaluated as intraoperative data. In postoperative evaluations, the extubation time, total drainage, blood transfusion, development of respiratory, renal, or left ventricular failure, revision due to bleeding, low cardiac output syndrome, intra-aortic balloon pump necessity, length of stay in the intensive care unit, length of hospital stay, development of atrial fibrillation, reintubation, exitus, myocardial infarction, acute cerebral hemorrhage, stroke or embolism, gastrointestinal bleeding, and respiratory distress were evaluated. The preoperative risk profile, intraoperative data, and postoperative results of octogenarians who underwent emergency OPCAB and ONCAB were compared.

The study protocol was approved by the Ministry of Health Mersin Provincial Health Directorate Local Ethics Committee (02.04.2019/27). The study was conducted in accordance with the principles of the Declaration of Helsinki. Informed consent was obtained from the participants.

Statistical Analysis

The Statistical Package for the Social Sciences version 21.0 for Windows (IBM Corp., Armonk, NY, USA) software package was used for data analysis. Categorical variables are presented as frequencies (n), and percentages (%), and numeric variables are presented as the mean ± standard deviation and median (minimum-maximum) value. The Kolmogorov-Smirnov test was used for normality analysis. The Chi-square test and Fisher's exact test were used to compare the distribution of categorical variables between groups. Independent-sample t-tests and the Mann-Whitney U test were used to compare continuous variables between two independent groups. A value of $p<0.05$ was accepted to be statistically significant.

Results

The analyses included 130 patients (56 women and 74 men) who underwent emergency OPCAB and 253 patients (86 women and 167 men) who underwent emergency bypass with the ONCAB procedure. The OPCAB and ONCAB groups are compared in terms of preoperative clinical features (Table I), operative data (Table II), and postoperative outcomes after CABG surgery (Table III).

The preoperative clinical features and statistical evaluation results of the demographic data are shown in Table I. In the preoperative evaluation, the blood urea nitrogen (BUN) and creatinine (Cr) values were significantly higher in the off-pump group than in the on-pump group. Although there was a statistical difference, the median values of BUN were very close to each other (BUN: 21 *vs.* 19; Cr: 1.1 *vs.* 1.1). In the preoperative echocardiographic evaluation, the left ventricular ejection fraction (EF) was lower in the off-pump group (EF median: 50% (min-max: 25-60) and 55% (min-max: 25-66), respectively; $p=0.020$). In the off-pump group, 4.6% (n: 6) and in the on-pump group, 2.4 % (n: 6) had preoperative EF ≤30. There was any statistically significant difference between the two groups in terms of poor left ventricular function before operation (Table I).

The two groups had similar intraoperative data, and there were no statistically significant differences between them (Table II).

Table III shows the significant differences in multiple postoperative variables between octogenarians undergoing OPCAB and ONCAB. ONCAB patients had a significantly longer ICU stay, longer hospital stay, a larger volume of transfused erythrocyte suspension, a higher proportion of low cardiac output syndrome and acidosis, a higher rate of acute renal failure (ARF), and a higher rate of stroke than OPCAB patients ($p=0.003$, $p=0.008$, $p=0.002$, $p=0.031$, $p=0.038$, and $p=0.022$). The median intensive care unit stays were longer in the ONCAB group rather than OPCAB, and the difference was statistically significant (1 *vs.* 1; $p=0.008$). The median hospital stays of the ONCAB and OPCAB groups were 7 (min 3-max 45) and 6 (min 4-max 40) days, and the difference was statistically significant on behalf of ONCAB ($p=0.003$). The median transfused ES units were 1 (min 0-max 13) and 1 (min 0-max 5). In addition, higher rates of ARF and stroke were observed in the ONCAB group than in the OPCAB group (ARF, mean: 10.7% *vs.* 4.6%; stroke, mean: 5.1% *vs.* 0.8%, respectively). Likewise, low cardiac output syndrome and acidosis were observed at a higher rate in the on-pump group (low cardiac output mean: 7.5% *vs.* 2.3%, and acidosis mean: 8.3% *vs.* 2.3%). There was no statistically significant difference between the groups in terms of mechanical ventilation time, inotropic support and intra-aortic balloon pump (IABP) requirement, mediastinitis, gastrointestinal system (GIS) complications, respiratory distress requiring reintubation, bleeding requiring re-exploration, total drainage amounts, atrial fibrillation, and mortality.

Discussion

According to our study, octogenarians who underwent emergency coronary bypass with a beating heart were less likely to have postoperative ARF and had a shorter intensive care unit stay and earlier discharge from the hospital. In octogenarians who underwent emergency on-pump coronary bypass procedures, postoperative stroke, low output syndrome, and metabolic acidosis were more common, and more blood transfusions were performed postoperatively.

In a study[26] comparing the incidence and severity of acute kidney injury in 1,612 elderly patients who had undergone off-pump and on-pump coronary bypass surgery, the preoperative kidney function was graded according to the glomerular filtration rate, and acute kidney injury (AKI) within the first week after surgery was defined and classified according to the Acute Kidney

Table I. Preoperative clinical data.

Variables	Off-pump (n: 130)	On-pump (n: 253)	p-value
Sex (male), % (n)	56.9 (74)	66 (167)	0.081†
Age **, years	84 (80-99)	85 (80-89)	0.108‡
Diabetes mellitus, % (n)	21.5 (28)	30.8 (78)	0.054†
Hypertension, % (n)	45.4 (59)	49 (124)	0.501†
COPD, % (n)	58.5 (76)	49 (124)	0.080†
Hyperlipidemia, % (n)	70.8 (92)	67.6 (171)	0.525†
Acute MI, % (n) % (n)	61.5 (80)	65.2 (165)	0.478†
Smoking, % (n)	56.9 (74)	58.5 (148)	0.767†
Drug use			
β-blockers, % (n)	36.9 (48)	41.1 (104)	0.428†
ACE inhibitor, % (n)	22.3 (29)	22.1 (56)	0.969†
Digoxin, % (n)	0.8 (1)	2 (5)	0.668§
ASA, % (n)	58.5 (76)	57.3 (145)	0.829†
Clopidogrel, % (n)	38.5 (50)	39.5 (100)	0.850†
LMCA, % (n)	48.5 (63)	41.9 (106)	0.221†
BUN, mg/dL	21 (11-63)	19 (7-58)	<0.001‡
Creatinine, mg/dL	1.1 (0.6-2.1)	1.1 (0.5-2.2)	0.037‡
Hematocrit, %	39.62±5.05	40.25±4.98	0.238¶
Ejection fraction, %	50 (25-60)	55 (25-66)	0.020‡
NYHA Class 0-1, % (n)	0.8 (1)	4 (10)	0.007†
NYHA Class 2, % (n)	94.6 (123)	83.4 (211)	0.944
NYHA Class 3-4, % (n)	4.6 (6)	12.6 (32)	0.013
With carotid endarterectomy, % (n)	3.1 (4)	4 (10)	0.665†
Preoperative AF, % (n)	1.5 (2)	2 (5)	1§
LVEF ≤30, % (n)	4.6 (6)	2.4 (6)	0.223

Data are presented as mean ± SD and numbers (%). **Non-normally distributed variables were presented as median (minimum-maximum) value. †: Chi-square test; ‡: Mann-Whitney U test; §: Fisher's exact test; ¶: Independent-sample *t*-tests were applied. COPD: Chronic obstructive pulmonary disease, MI: Myocardial infraction, ACE: Angiotensin converting enzyme, ASA: Acetylsalicylic acid, LMCA: Left Main Coronary Artery, BUN: Bood urea nitrogen, NYHA: New York Heart Associated, AF: Atrial fibrillation, LVEF: Left ventricular ejection fraction.

Injury Network (AKIN) criteria. According to this study[26], the incidence rates of AKI were similar for elderly patients undergoing off-pump and on-pump CABG, and surgical techniques had no detectable effect on the incidence or severity of AKI in elderly patients with preexisting kidney disease. In another study, which included 466 patients aged 70 years and older who underwent isolated CABG surgery and underwent on-pump and off-pump surgery, the effects of AKI in both procedures on the need for dialysis and mortality were compared; AKI developed in 40.3% of all patients, and 2.3% required dialysis. Compared with on-pump CABG, off-pump CABG was not associated with either a decrease in postoperative AKI rate or severity or a reduction in the long-term new onset of dialysis or mortality[27]. In a different study[28] that retrospectively

Table II. Intraoperative data.

Variables	Off-pump (n: 130)	On-pump (n: 253)	p-value
Left internal mammary artery, % (n)	85.4 (111)	86.2 (218)	0.835†
Cross clamp time **, min	-	51 (12-137)	-
Total CPB time **, min	-	85 (0-163)	-
Per-operative MI, % (n)	1.5 (2)	3.6 (9)	0.263†
Grafts **, n	3 (1-4)	3 (1-4)	0.504‡
Left internal mammary artery, % (n)	85.4 (111)	86.2 (218)	0.835†
Cross clamp time **, min	-	51 (12-137)	-
Total CPB time **, min	-	85 (0-163)	-

Data are presented as mean ± SD and numbers (%). **Non-normally distributed variables were presented as median (minimum-maximum) value. †: Chi-square test; ‡: Mann-Whitney U tests were applied. CPB: Cardiopulmonary bypass, MI: Myocardial infraction.

Table III. Postoperative outcome.

Variables	Off-pump (n: 130)	On-pump (n: 253)	p-value
Reoperation due to bleeding, % (n)	2.3 (3)	3.6 (9)	0.506†
Postoperative AF, % (n)	17.7 (23)	13.8 (35)	0.319†
Stroke, % (n)	0.8 (1)	5.1 (13)	0.031†
Mortality, % (n)	31 (4)	4 (10)	0.665†
Low cardiac output, % (n)	2.3 (3)	7.5 (19)	0.038†
Acidosis, % (n)	2.3 (3)	8.3 (21)	0.022†
Need for inotropic agents, % (n)	11.5 (15)	16.6 (42)	0.187†
Reintubation, % (n)	2.3 (3)	5.1 (13)	0.190†
Acute kidney disease, n% (n)	4.6 (6)	10.7 (27)	0.045†
IABP, % (n)	4.6 (6)	8.3 (21)	0.182†
Detachment, % (n)	0 (0)	0.8 (2)	0.550§
Post-perfusion mind, % (n)	0 (0)	1.6 (4)	0.304§
GIS complication, % (n)	0 (0)	1.2 (3)	0.554§
Drainage**, cc	500 (50-1,750)	450 (50-3,000)	0.500‡
Extubation time**, hours	8 (2-19)	8 (3-75)	0.495‡
Intensive care unit stay**, days	1 (1-5)	1 (1-45)	0.008‡
Total hospitalization stay**, days	6 (4-40)	7 (3-45)	0.003‡
Blood product use**, packages	1 (0-5)	1 (0-13)	0.002‡

Data are presented as mean ± SD and numbers (%), **Non-normally distributed variables were presented as median (minimum-maximum) value. †: Chi-square test; ‡: Mann-Whitney U test; §: Fisher's exact test was applied. AF: Atrial fibrillation, IABP: Intra-aortic balloon pump, GIS: Gastrointestinal system.

analyzed the clinical data of 2,242 patients who underwent CABG, including patients with AKI after CABG, advanced age, preoperative chronic renal dysfunction, on-pump CABG, postoperative respiratory dysfunction, and low cardiac output syndrome were reported to be risk factors for AKI after CABG. In fact, the largest single randomized trial[29] of off-pump coronary surgery (CORONARY) showed an absolute risk reduction of 4.1% in any AKI defined according to the AKIN criteria in patients undergoing off-pump CABG. In our study, although preoperative renal function tests were higher in octogenarians who underwent off-pump emergency bypass, ARF requiring postoperative dialysis was more common in the octogenarian group who underwent on-pump emergency bypass. The organ-damaging effect of CPB, which is a well-known fact, was also reflected in the results of our study. Our results show that the risk of ARF, which is one of the most important causes of postoperative mortality and morbidity, can be reduced by the off-pump bypass method, even in advanced-age patients.

In our study, blood product transfusion was often required for elderly individuals who underwent on-pump emergency bypass. The increased need for blood transfusion after cardiac surgery is a predetermined indicator of poor outcomes[30-32]. In addition, a large meta-analysis showed that isolated CABG operations were more likely to require blood transfusions in on-pump patients than in off-pump patients, which has been attributed to the inflammatory response of CPB[33]. Although the amount of postoperative drainage was not different in our study, the need for blood product transfusion increased in octogenarians who underwent on-pump emergency coronary bypass compared to those who underwent off-pump bypass, which is consistent with the current literature, but this increase did not affect the mortality rates.

Studies[34-38] have shown that stroke rates after CABG were between 0.5 and 3.1 per 100 cases. The occurrence of such adverse events in patients with CABG indicates a poor prognosis in terms of morbidity and mortality. In a study that analyzed almost 670,000 patients from a nationwide inpatient sample from 1999 to 2011, the stroke rate was 1.9%, with 2.1% mortality and 49% morbidity, which led to a 5-fold increased risk of operative death and complications38. Age, chronic renal failure, recent myocardial infarction, previous cerebrovascular accident, hypertension, diabetes mellitus, left ventricular dysfunction, low cardiac output syndrome, atrial fibrillation, cerebrovascular disease, and the use of an intra-aortic balloon pump were shown[39] to increase the risk of postoperative neurological events. All patient groups in our study had all these risk factors and underwent emergency surgery, in

accordance with the literature. In a comparative meta-analysis[40] of studies involving 27,623 patients with on-pump and off-pump coronary artery bypass grafting for octogenarians, off-pump CABG had a lower hospital mortality rate, a lower stroke rate, and a shorter hospital stay than on-pump CABG, which was associated with an economic burden. Interestingly, in the literature, it was also reported that there was no difference in postoperative stroke between off-pump and on-pump CABG and that off-pump surgery reduced the incidence of stroke, especially in elderly patients. Manipulation of the aorta is significantly less in OPCAB surgery. Aortic graft anastomosis is performed with a side-clamp in OPCAB surgery to minimize the risk of embolization of atheromatous plaques in the aorta. Atherosclerosis in the ascending aorta is an important risk factor for stroke, which increases with age. The potential advantages of OPCAB are more evident in patients with stroke risk factors such as calcified aorta, previous stroke history, hypertension, and peripheral arterial diseases. For this reason, the benefits of OPCAB in terms of stroke highlight outcomes better in subgroups, including elderly patients, compared to patients with low stroke risk. In our study, the incidence of stroke was found to be lower in octogenarians who underwent emergency coronary bypass surgery with both techniques compared to the group who underwent OPCAB. Considering our study results, we think the stroke rates will be lower if emergency coronary bypass surgery is performed with the off-pump method in elderly patients with a high risk of stroke.

In a comparative meta-analysis41 of 54 studies on 16,261 patients, it was stated that with off-pump CABG, the ventilation times, length of stay in the intensive care unit and hospital, and health care costs could be reduced. Another study[42] reported that hospital costs were 20% higher in older patients, which was partly attributed to an average length of stay of 3.9 days compared to younger patients. Unlike on-pump CABG, off-pump CABG has a shorter hospital stay in octogenarians who underwent emergency bypass surgery in our study, which is consistent with the meta-analysis and offers the advantages of a lower hospital stroke rate and shorter hospital stay to octogenarians40. With this result, the cost of health has clearly decreased, which is consistent with the aforementioned studies[34-42]. In addition, we think that the shorter hospital stay for off-pump CABG patients may be partially attributed to the lower stroke rate. Postoperative stroke requires long-term institutional care, which translates into prolonged hospital stays and a significant economic burden.

Age and comorbidity in cardiac surgery are extremely effective in the success of surgery. When CABG studies are examined with subgroup analyses, factors such as age and kidney failure cause differences in the outcomes of the group to which it belongs. At the same time, emergency cardiac surgery is a clinical situation that most clinicians do not volunteer for. Octogenarians presenting with ACS, located in the intersection cluster of both risky conditions, burden many surgeons. In our study, the results of OPCAB and ONCAB were compared in this group of patients who were at high risk. The OPCAB group, which allowed less manipulation of the heart, was superior in preventing major complications, even at the expense of complete revascularization.

Limitations

Our study is limited by the fact that it is retrospective. It also reflects the nature and experience of a single institution. In addition, our results regarding mortality and morbidity are limited to in-hospital events, as follow-up results regarding long-term survival were unavailable.

Conclusions

Although we found that the effect of the related surgical technique on mortality was neither advantageous nor disadvantageous, our study comparing emergency OPCAB *vs.* emergency ONCAB in octogenarians showed that as a revascularization option in elderly patients with acute coronary syndrome, emergency OPCAB better prevented major complications. However, those benefits should be tested in larger cohorts and tailored randomized studies.

Ethics Approval

The present study was approved by the Local Ethics Committee of the Republic of Turkey Ministry of Health Mersin Provincial Health Directorate (Reference number: 001-27).

Informed Consent

Informed consent was obtained from the participants.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Authors' Contributions

EET, BA, MAY, and DO designed the research study. EET performed the research. MAY and DO analyzed the data. EET, MAY, and DO wrote the manuscript.
All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

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