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Reliability of multi-vessel off-pump coronary artery by-pass surgery in patients who have low risk profile

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Abstract

We investigated the reliability of multi-vessel off-pump coronary artery by-pass surgery in patients who have low risk profile. This study included 53 patients who undergone off-pump coronary artery by-pass surgery. Patients have been allocated two groups in terms of graft count. 1 and 2 vessel patients were defined as Group A and 3,4,5 vessel patients as Group B. Baseline and serial post-operative CK-MB values were obtained. Ejection fraction (LVEF), mitral-myocardial systolic velocities (lateral S' and septal S'), mean E', left ventricle filling pressure index (E/E' ratio) and tricuspid-S' were calculated at pre and postoperative period. Intensive care unit stay time was moderately correlated with pre mean E' (r = -0.32, p=0.020) and pre E/E' ratio (r = 0.34, p=0.013). Δ CK-MB values were similar between two groups (p=0.263). There are no differences between groups in terms of Δ LVEF, Δ mean E', Δ lateral S' and Δ tricuspid S'. Δ E/E' ratio was mildly in favour of group A patients (-2.31 ± 2.70 vs. -0.29 ± 2.89, p=0.007). Furthermore, Δ septal S' was slightly in favour of group A patients (0.71 ± 2.39 cm/s vs. -0.66 ± 1.73 cm/s, p=0.017). Multi-vessel off-pump coronary artery by-pass surgery seems as safe as 1 and 2 vessels despite small differences in terms of cardiac function alterations in patients who have low risk profile.

Keywords: Multi-vessel off-pump coronary artery surgery, reliability, low risk profile

Introduction

Off-pump coronary artery bypass (OPCAB) graft surgery has been assessed as a safe alternative to conventional coronary artery bypass graft surgery with cardiopulmonary bypass (ONCAB) for myocardial revascularization since it may be associated with decreased postoperative morbidity [1,2]. Perioperative myocardial ischemia is an important complication, increasing mortality and morbidity after cardiac surgery [3]. Clinical trials revealed that the release of troponin I following OPCAB is lower than after ONCAB [4]. A major pitfall to OPCAB was difficulty of performing coronary anastomoses with the needed precision, particularly on the lateral and posterior surfaces of the heart. Recent improvement in the technology has allowed multiple-vessel grafting in all coronary areas using OPCAB possible with a graft patency comparable to ONCAB [2].

The aim of our study was to investigate reliability of multi-vessel off-pump coronary artery by-pass surgery in patients who have low risk profile using close cardiac enzyme monitoring and prepostoperative detailed echocardiographic parameters in terms of cardiac function alterations.

Material and Methods

Fifty-three patients who have undergone off-pump coronary artery by-pass surgery were included in this study. Patients were divided into two groups in terms of used graft count at the operation. 1 and 2 vessels were defined group A and 3,4 and 5 vessels were defined group B. The following patients were excluded from this study: severe morbid obesity [body mass index (BMI) > 35], moderate or severe heart valve insufficiency and/or stenosis, congenital heart disease, atrial fibrillation, left bundle branch block, established myocardial infarction within last one week, left ventricular ejection fraction (LVEF) < 40 %, symptomatic heart failure, poor echocardiographic image quality, stroke, chronic obstructive pulmonary disease, chronic renal failure or musculoskeletal disorder.

All study patients underwent a thorough clinical, anthropometric, and laboratory investigation. Participants were classified as hypertensive if resting systolic blood pressure was \geq 140mmHg and/or diastolic blood pressure was \geq 90 mmHg or if they took antihypertensive medications. Diabetes was diagnosed according to World Health Organization criteria [5]. Smoking history was defined as any current or past form of tobacco use. Height and weight of all subjects were measured and the body mass index (BMI) was calculated as weight (kg) divided by height² (m). Blood samples were obtained one day ago from the operation. All patients

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had serial measurement of CK-MB values at the following times: preoperatively at 6, 12, 18, 24, 48h after the end of the operation. The postoperative highest value of CK-MB was defined peak CK-MB. Δ CK-MB was calculated as peak CK-MB minus baseline CK-MB.

Operative technique: The heart was approached via standard median sternotomy. Normothermia was ensured during the operations by warming the operative theatre and using a heating mattress. A single no:1 silk suture, which had been passed previously through a gauze swab, was placed in the posterior pericardium at the midline between the inferior vena cava and the left inferior pulmonary vein. This retraction stitch enabled vertical displacement of the cardiac apex. Intravenous heparin (80 IU/kg) was administered prior to beginning the distal anastomoses with a target activated clotting time between 250-300 s. The distal anastomosis of left internal mammary artery (LIMA) on left anterior descending (LAD) was performed first. Proximal anastomoses of venous or free arterial grafts were made before performing distal anastomoses. A tissue stabilizer (ACROBAT-i Stabilizer System - Maquet) was used in all patients. Distal anastomoses were performed by the aid of a CO2-water blower (Clearview Medtronic, Inc., Minneapolis, MN). Anastomosis time was defined as sum of each distal anastomosis time. Operation time was defined from skin to skin time. Patients who were taken intensive care unit (ICU) at postoperative period were discharged to service in case of extubation and hemodynamic stabilization.

Echocardiographic measurements: Each patient underwent standard transthoracic two- dimensional echocardiography one day ago from the operation. Post-operative echocardiographic parameters were taken between 48h and 72h after operation. Patients who developed atrial fibrillation were treated with infusion. Post-operative echocardiographic amiodarone parameters were taken after sinus rhythm was restored for these patients. All echocardiographic measurements were performed by one operator, who was blinded for the clinical and laboratory results of the study group. GE Vivid S5 cardiovascular ultrasound machine (GE, Vingmed, Horten, Norway) with a 2,5 MHz phasedarray transducer was used for each study subject. End-diastolic volume (EDV), end-systolic volume (ESV) and ejection fraction (EF) was calculated using the biplane Simpson's method.

From the apical four-chamber view, pulse-wave Doppler recordings of the mitral inflow were obtained with the sample volume placed at the tips of the mitral valve leaflets. Peak velocities of early (E) was measured by pulse-wave Doppler. All Doppler signals were recorded with a chart recorder set at 100 mm/sec. The averages of 3 cycles were used.

The TDI program was set to pulse-wave Doppler mode. Filters were set to exclude high frequency signals. Gains were minimized to allow a clear tissue signal with minimal background noise. The TDI of the diastolic and systolic velocities was obtained from the apical 4-chamber view. A 1.5-mm sample volume was placed at the lateral corner of mitral, septal corner of mitral and lateral corner of tricuspid annulus. Analysis was performed for early (E') and myocardial systolic velocity (S'). Mean E' was calculated as septal E' plus lateral E' divided by 2 for diastolic performance of left ventricle. In addition, E/E' (mean E') was calculated as an indicator of left ventricle filling pressure. Left ventricular

systolic function was evaluated with lateral S' and the septal S'. Right ventricular systolic function was assessed with tricuspid S' since these parameters are relatively less effected from the preload condition. The values of Δ were calculated as post-operative value minus pre-operative value for all echocardiographic parameters.

Statistical Analysis

The distributions of continuous variables were evaluated using Shapiro-Wilk test. The results with distribution were expressed as mean \pm standard deviation. Categorical variables were expressed as percentages. Statistical analyses were performed using SPSS for Windows version 23.0. For continuous variables, the differences between two groups were compared using Mann Whitney U-test for non-parametric data. Pre-post comparisons were done with Wilcoxon test for paired samples. Categorical parameters were analyzed using χ^2 test. Correlation was assessed using Spearman's correlation coefficient. p< 0.05 was considered as statistically significant for all tests.

Results

Fifty-three patients (mean age 61.00 ± 8.78 years) were included in this study. Thirty-eight patients (71.7%) have hypertension diagnosis and twenty patients (37.7%) have diabetes mellitus diagnosis. Twenty-nine patients (54.7%) were smoker. Atrial fibrillation has occurred in ten patients (18.9%) at postoperative period. According to graft count, six patients (11.3%) have 1, fifteen patients (28.3%) have 2, twenty-three patients (43.4%) have 3, four patients (7.5%) have 4 and five patients (9.4%) have 5. LIMA-LAD anastomosis was used for all patients. Saphenous vein grafts were usually used for non-LAD anastomosis. Arterial grafts were used only five patients (9.4%) for non-LAD anastomosis. For all group, mean ICU stay time was assessed $1362.64 \pm$ 429.61 minutes, mean operation time was calculated 116.17 \pm 28.05 minutes and mean anastomosis time was measured 49.06 \pm 25.42 minutes. Mean CK-MB value of all group has significantly increased at postoperative period (1.42 \pm 0.79 ng/dl vs. 39.36 \pm 56.31 ng/dl, p<0.001).

Pre-op and post-op echocardiographic parameters of all patients are presented in Table-1. LVEF has decreased mildly but statistically significant at postoperative era (p=0.001). Mean E' has increased (p=0.046) and E/ E' ratio has decreased (p=0.009) at postoperative period. For tissue Doppler velocities, only tricuspid S' value has changed in favour of decreasing (p<0.001).

The Spearman's correlation analysis that was including graft count, age, BMI, hypertension, diabetes mellitus, smoking, atrial fibrillation, LDL-cholesterol, creatinine, potassium, hematocrit, Δ CK-MB, anastomosis time, operation time, ICU stay time and echocardiographic parameters was performed on the all patients. There is a mild correlation between graft count and Δ E/E' ratio (r = 0.27, p=0.050). Furthermore, there are negative correlations between graft count and Δ Septal S' (r = -0.33, p=0.015) and Δ Tricuspid S' (r = -0.31, p=0.022). As expected, there are strong correlations between graft count and anastomosis time (r = 0.90, p<0.001) and operation time (r = 0.77, p<0.001). We have detected a significant correlation between atrial fibrillation incidence and pre E/E' ratio (r = 0.33, p=0.017). ICU stay time was moderately correlated with pre mean E' (r = -0.32, p=0.020) and pre E/E' ratio (r = 0.34, p=0.013) (Figure-1). Operation time was correlated with

 Δ CK-MB (r = 0.28, p=0.043) and Δ E/ E' ratio (r = 0.31, p=0.024). Δ CK-MB was correlated with Δ EF (r = -0.41, p=0.002) and Δ E/ E' ratio (r = 0.39, p=0.003).

Echocardiographic parameters	Pre-Op (n=53)	Post-Op (n=53)	Р
EDV (mL)	108.79 ± 32.01	93.04 ± 24.96	< 0.001
ESV (mL)	43.32 ± 18.84	40.51 ± 15.24	0.097
LVEF (%)	61.17 ± 6.58	57.08 ± 7.57	0.001
Mean E' (cm/s)	6.81 ± 1.78	7.34 ± 1.79	0.046
E/ E' ratio	10.59 ± 2.86	9.50 ± 3.39	0.009
Lateral S' (cm/s)	7.58 ± 2.17	7.25 ± 2.06	0.338
Septal S' (cm/s)	7.36 ± 1.66	7.25 ± 1.90	0.621
Tricuspid S' (cm/s)	13.19 ± 3.04	9.30 ± 2.04	< 0.001

EDV; left ventricular end-diastolic volume, ESV; left ventricular end-systolic volume, LVEF; left ventricular ejection fraction, Mean E'; mean of mitral annular peak diastolic E' velocities, Lateral S'; mitral lateral annulus peak myocardial systolic velocity, Septal S'; mitral septal annulus peak myocardial systolic velocity, Tricuspid S'; tricuspid lateral annulus peak myocardial systolic velocity

 Table 2. Baseline clinical, biochemical and operation parameters of subgroup patients

Variables	Group A (n=21)	Group B (n=32)	Р
Age, (year)	62.24 ± 8.97	60.19 ± 8.70	0.413
Male, n (%)	17 (81)	28 (87.5)	0.698
Diabetes mellitus, n (%)	8 (38.1)	12 (37.5)	0.965
Hypertension, n (%)	15 (71.4)	23 (71.9)	0.972
Smoking, n (%)	8 (38.1)	21 (65.6)	0.049
AF incidence, n (%)	3 (14.3)	7 (21.9)	0.722
Graft Count, n	1.71 ± 0.46	3.44 ± 0.76	< 0.001
BMI (kg/m ²)	28.25 ± 3.32	29.12 ± 4.77	0.630
Hematocrit (%)	40.31 ± 3.92	40.53 ± 3.74	0.928
Serum creatinine (mg/dl)	0.82 ± 0.11	0.86 ± 0.21	0.696
Potassium (mEq/l)	4.48 ± 0.36	4.26 ± 0.26	0.008
LDL-Cholesterol (mg/dl)	111.94 ± 32.39	123.78 ± 31.58	0.220
Baseline CK-MB (ng/dl)	1.45 ± 0.82	1.40 ± 0.78	0.834
Peak CK-MB (ng/dl)	27.06 ± 30.47	47.43 ± 67.45	0.203
Anastomosis time (minute)	26.10 ± 13.36	64.13 ± 19.42	< 0.001
Operation time (minute)	93.29 ± 24.19	131.19 ± 18.84	< 0.001
ICU stay time (minute)	1339.05 ± 120.82	1378.13 ± 547.31	0.220

BMI; Body Mass Index, CK; Creatine Kinase, ICU; Intensive care unit

Baseline clinical, biochemical and operation parameters of subgroup patients are presented Table-2. Mean graft count of group A and group B was different (1.71 ± 0.46 vs. 3.44 ± 0.76 , p<0.001). Group B patients had lower potassium level compared to group A patients despite similar serum creatinine levels. Higher smoking rates were observed in group B. As expected, anastomosis time (p<0.001) and operation time (p<0.001) was longer in Group B patients. Group B patients have higher Δ CK-MB values than group A but this difference did not reach statistical meaning

(p=0.263) (Figure-2). Change of echocardiographic parameters in Group A patients are presented Table-3. Value of LVEF was decreased mildly in postoperative period (60.86 ± 6.69 vs. $56.57 \pm$ 8.63, p=0.025). E/E' ratio has improved slightly $(10.90 \pm 2.67 \text{ vs.})$ 8.59 ± 2.77 , p=0.002). Only tricuspid S' value of tissue Doppler velocities has changed in favour of mildly decreasing (11.90 ± 2.91) cm/s vs. 8.90 ± 2.30 cm/s, p=0.001). Change of echocardiographic parameters in Group B patients are presented Table-4. LVEF was decreased mildly in postoperative period (61.38 ± 6.61 vs. $57.41 \pm$ 6.92, p=0.015). Values of septal S' and tricuspid S' have decreased slightly [$(7.78 \pm 1.75 \text{ cm/s vs. } 7.13 \pm 1.86 \text{ cm/s, } p=0.042)$ and $(14.03 \pm 2.87 \text{ cm/s vs. } 9.56 \pm 1.85 \text{ cm/s, } p<0.001)$]. Comparison of changes on echocardiographic parameters in subgroup patients are presented Table-5. There is no difference between subgroups in term of values of Δ LVEF (p=0.848). Δ E/E' ratio was mildly in favour of group A patients $(-2.31 \pm 2.70 \text{ vs.} -0.29 \pm 2.89, p=0.007)$. Furthermore, Δ septal S' was slightly in favour of group A patients $(0.71 \pm 2.39 \text{ cm/s vs.} -0.66 \pm 1.73 \text{ cm/s, p}=0.017).$

Table 3. Change of echocardiographic parameters in Group A patients

Echocardiographic parameters	Pre-Op (n=21)	Post-Op (n=21)	Р
EDV (mL)	105.33 ± 38.83	90.00 ± 30.59	0.002
ESV (mL)	42.67 ± 23.44	39.52 ± 17.45	0.243
LVEF (%)	60.86 ± 6.69	56.57 ± 8.63	0.025
Mean E' (cm/s)	6.35 ± 1.23	7.11 ± 1.78	0.106
E/ E' ratio	10.90 ± 2.67	8.59 ± 2.77	0.002
Lateral S' (cm/s)	7.24 ± 2.46	7.62 ± 2.26	0.452
Septal S' (cm/s)	6.71 ± 1.31	7.43 ± 1.99	0.221
Tricuspid S' (cm/s)	11.90 ± 2.91	8.90 ± 2.30	0.001

EDV; left ventricular end-diastolic volume, ESV; left ventricular end-systolic volume, LVEF; left ventricular ejection fraction, Mean E'; mean of mitral annular peak diastolic E' velocities, Lateral S'; mitral lateral annulus peak myocardial systolic velocity, Septal S'; mitral septal annulus peak myocardial systolic velocity, Tricuspid S'; tricuspid lateral annulus peak myocardial systolic velocity

Table 4. Change of echocardiographic parameters in Group B patientss

Echocardiographic parameters	Pre-Op (n=32)	Post-Op (n=32)	Р
EDV (mL)	111.06 ± 27.06	95.03 ± 20.77	< 0.001
ESV (mL)	43.75 ± 15.50	41.16 ± 13.86	0.213
LVEF (%)	61.38 ± 6.61	57.41 ± 6.92	0.015
Mean E' (cm/s)	7.11 ± 2.04	7.50 ± 1.81	0.274
E/ E' ratio	10.40 ± 2.99	10.10 ± 3.67	0.601
Lateral S' (cm/s)	7.81 ± 1.98	7.00 ± 1.92	0.061
Septal S' (cm/s)	7.78 ± 1.75	7.13 ± 1.86	0.042
Tricuspid S' (cm/s)	14.03 ± 2.87	9.56 ± 1.85	< 0.001

EDV; left ventricular end-diastolic volume, ESV; left ventricular end-systolic volume, LVEF; left ventricular ejection fraction, Mean E'; mean of mitral annular peak diastolic E' velocities, Lateral S'; mitral lateral annulus peak myocardial systolic velocity, Septal S'; mitral septal annulus peak myocardial systolic velocity, Tricuspid S'; tricuspid lateral annulus peak myocardial systolic velocity

Table 5. Comparison of changes on echocardiographic parameters in subgroup patients

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Echocardiographic parameters	Pre-Op (n=21)	Post-Op (n=32)	Р
Δ LVEF (%)	$\textbf{-4.29} \pm 7.04$	-3.97 ± 8.13	0.848
Δ Mean E' (cm/s)	0.76 ± 1.95	0.39 ± 1.82	0.380
Δ E/ E' ratio	$\textbf{-2.31} \pm 2.70$	$\textbf{-}0.29\pm2.89$	0.007
Δ Lateral S' (cm/s)	0.38 ± 2.50	$\textbf{-0.81} \pm 2.33$	0.089
Δ Septal S' (cm/s)	0.71 ± 2.39	$\textbf{-0.66} \pm 1.73$	0.017
Δ Tricuspid S' (cm/s)	-3.00 ± 2.95	-4.06 ± 3.27	0.161

LVEF; left ventricular ejection fraction, Mean E'; mean of mitral annular peak diastolic E' velocities, Lateral S'; mitral lateral annulus peak myocardial systolic velocity, Septal S'; mitral septal annulus peak myocardial systolic velocity, Tricuspid S'; tricuspid lateral annulus peak myocardial systolic velocity

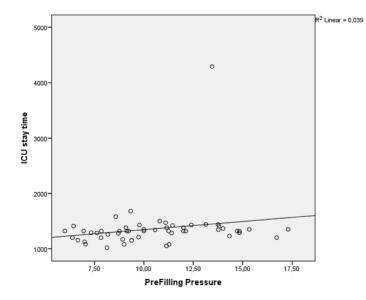


Figure 1. Correlation between ICU stay time and pre E/E' ratio (filling pressure index)

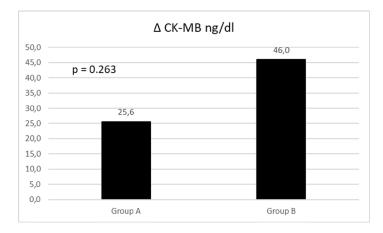


Figure 2. Comparison of \triangle CK-MB values in subgroup patients

Discussion

Given that for all population we found that, there is not significant alteration on early postoperative left ventricular systolic functions since lateral S' and septal S' values do not change although LVEF value mildly decrease. Furthermore, we found that, early revascularization has improved early left ventricular diastolic function and filling pressure by taking these results into account for improvement of mean E' and E/ E' ratio values at postoperatively.

Since cardiopulmonary bypass is avoided, OPCAB has superiority in myocardial protection compared with ONCAB [6]. It has been reported that OPCAB is associated with better myocardial energy preservation, less oxidative stress, and minimal myocardial damage [7]. There are several randomized and prospective studies as well as low level of evidence studies supporting the clinical advantages of OPCAB in reducing myocardial infarction rate compared with that of ONCAB [8]. Besides, in some studies, there was no striking difference in the incidence of postoperative myocardial infarction between ONCAB and OPCAB [9]. However, the release of myocardial enzyme including troponin I has been declared to be reduced after OPCAB compared to ONCAB [4]. The elimination of traditional myocardial protection strategies such as cardioplegia and the hypothermia and accumulated myocardial dysfunction associated with hemodynamic instability or coronary flow interruption during coronary anastomosis raises the need for myocardial protection to preserve myocardial function during OPCAB [10]. Therefore, protecting the myocardium from ischemia is still one of the main problems cardiovascular surgeons have faced. The main question in our mind was whether or not myocardial dysfunction increase as number of graft count step up. It seems that increased graft count number does not affect myocardial functions harmfully by taking these results into account for no differences Δ CK-MB, Δ Mean E', Δ Lateral S', Δ Tricuspid S' and Δ LVEF values between subgroups despite Δ E/E' ratio and Δ septal S' values in slightly favour of group A patients.

We have reached another important data in terms of right ventricular systolic function. The values of tricuspid S' have decreased mildly postoperative period for all patients. There is no difference between subgroups in terms of Δ Tricuspid S' despite mild negative correlation between graft count and Δ Tricuspid S'. Right ventricular dysfunction after cardiac surgery is a well-known phenomenon that can be seen immediately after cardiac surgery. Both contraction and filling of the right ventricular are impaired after coronary by-pass graft surgery, however, the mechanism of this phenomenon is not yet understood exactly. It may be result from intraoperative ischemia, intraoperative myocardial damage and the use of cardiopulmonary pump [11,12]. Several clinical trials have shown that intraoperative right ventricle dysfunction can last up to 6 months after the surgery [13]. Some other studies have reported that the echocardiographic right ventricular dysfunction can last up to even 1 year after CABG [14,15] although some studies have detected a minor clinical value for such disorders [14]. Recently, Khani M et al have shown reversible right ventricular systolic function which is improved after three months from operation in patients who have undergone OPCAB [16]. In addition to literature, our findings have shown that increased graft count may affect slightly negative right ventricular systolic function despite no difference between subgroups.

We have found important finding about correlation between atrial fibrillation incidence and pre E/ E' ratio. A meta-analysis demonstrates important evidence that OPCAB reduces the occurrence of atrial fibrillation with a 20% RR reduction compared

to ONCAB [17]. Advanced age, arterial hypertension, obesity, male sex, abrupt withdrawal of beta blockers, and diabetes mellitus were also detected to be predisposing factors of postoperative supraventricular arrhythmias [18]. Depressed left ventricular systolic function has also been underlined as an important factor for the AF after conventional CABG surgery [19]. It has been shown that aging and hypertension results in major cardiovascular changes, including decreased elasticity and compliance of the aorta and great arteries [20]. These alterations produce a higher systolic pressure, increased resistance to left ventricular ejection fraction, and subsequent mild LVH and fibrosis. It has been demonstrated that left ventricular early diastolic filling progressively slows after the age of 20 years, so by 80 years the rate is reduced on an average up to 50% [21]. Increased fibrosis of left ventricular myocardium is a mechanism for the reduced filling rate arising in the early diastolic phase. More ventricular filling occurs in late diastole as the result of a more vigorous atrial contraction. The augmented atrial contraction is accompanied with atrial hypertrophy at an earlier stage and atrial enlargement at a later one, creating in this way a susceptible substrate for the genesis of reentry arrhythmias. All of these physiopathological changes may be represented by E/ E' ratio which has been shown to be the most accurate and relatively preload-independent noninvasive predictor of LV filling pressure. This ratio has demonstrated excellent reproducibility, with an E/E' ratio >15 found to be the best Doppler predictor of increased mean LV diastolic pressure and pulmonary artery wedge pressure [22]. Recently, some studies have found that increased LV filling pressure, assessed by E/E' ratio, is an independent predictor of 30-day and 1-year MACE in patients who undergo elective offpump coronary artery bypass graft surgery [23,24]. Furthermore, we have found that ICU stay time was moderately correlated with pre mean E' and pre E/E' ratio. These findings have underlined importance of that the diastolic function and LV filling pressure values of patients at preoperative period may affect postoperative hemodynamic stabilization at intensive care unit. Since there is no correlation graft count and atrial fibrillation incidence and no difference of atrial fibrillation incidence between subgroups, graft count number does not seem to affect development of atrial fibrillation. Given that these valuable data, E/E' ratio is important predictor for patients who undergone OPCAB even in situation of preserved ejection fraction and low risk profile.

Conclusion

In conclusion, multi-vessel off-pump coronary artery by-pass surgery seems as safe as 1 and 2 vessels despite small differences in terms of cardiac function alterations in patients who have low risk profile.

Limitation of the study

Patients may have been allocated to specific groups for each graft count if study population number had been sufficient. Then, more specific data may have been obtained in relation to graft count and change of cardiac functions. Another limitation of this study is that we could not obtained post-operative late period echocardiographic parameters. We may have shown whether or not these changes of cardiac function are permanent if we had got these data.

Competing interests

The authors declare that they have no competing interest

Financial Disclosure

The financial support for this study was provided by the investigators themselves.

Ethical approval

Before the study, permissions were obtained from local ethical committee.

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