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# Cardiovascular Function during First 24 Hours after Off-Pump and On-Pump CABG—A Prospective Observational Comparative Study

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## Abstract

**Purpose:** Myocardial revascularization by coronary artery bypass grafting (CABG) in ischemic heart disease patients has direct impact on hemodynamic parameters in the immediate post-operative period. The peri-operative cardiovascular functioning is an important determinant for outcome of surgery. In On-Pump CABG (ONCAB), the cardiopulmonary bypass has a negative effect on myocardium. Off-Pump CABG (OPCAB) avoids the effect of CPB but complete revascularization with difficult positioning of heart is technically demanding. This study is aimed to compare the cardiovascular functioning in the immediate post-operative period after OPCAB and ONCAB. **Methods:** Total 106 patients were operated for CABG from January 2015 to June 2016, of which 75 patients were operated for OPCAB and 31 patients were operated for ONCAB. For the comparison, hemodynamic parameters were measured during anesthesia before surgery, postoperatively after 1 and 4 hours (h) in the ICU, and in the morning after surgery, approximately after 20 h. **Results:** The time-dependent rise of hemodynamic parameters like Cardiac Output (CO), Cardiac Index (CI), Stroke volume (SV) and Left Ventricular Stroke Work Index (LVSWI) in the immediate post-operative hours (1 h and 4 h) are more predominant in OPCAB group than ONCAB group although the difference is eliminated mostly at 20 h. The better peripheral vasodilation after OPCAB causes immediate fall of Systemic Vascular Resistance Index (SVRI) after OPCAB. **Conclusion:** Better cardiovascular functioning immediately after OPCAB than ONCAB may be important for better hemodynamic stability. The difference is however eliminated after 24 hours indicating little significance in long term outcome.

## Keywords

Off-Pump CABG, On-Pump CABG, Cardiac Index, Stroke Volume, Left

Ventricular Stroke Work Index, Systemic Vascular Resistance Index

## 1. Introduction

Coronary artery bypass grafting was initially started as a beating heart surgery for myocardial revascularization in coronary artery diseases. After invention of the cardiopulmonary bypass (CPB), it was used during surgery for the ease of anastomosis in difficult locations and positions of the heart without any hemodynamic instability or adverse events. However, soon it was realized that the cascade of inflammatory responses generated by extracorporeal circulation can have negative effect on myocardium and other organs which is further worsened by aortic cross-clamping and cardioplegic arrest [1] [2]. There is a resurgence of OPCAB procedure due to the advantage of avoidance of CPB especially for the patients with compromised renal function or cerebrovascular diseases. The debate continues regarding the superiority of OPCAB over ONCAB due to the technical difficulty to achieve complete myocardial revascularization in off-pump beating heart surgery, particularly in multiple vessels involvement due to extensive coronary artery disease [1]. In the current era, with the substantial progress in the field of cardiac anesthesia and surgical techniques and equipment, OPCAB has become a routine procedure across the world even in patients with multi-vessel diseases with comparable results to conventional CABG [2] [3] [4] [5]. Although there are many comparative studies between OPCAB and ONCAB, there is paucity of literature regarding the immediate hemodynamic effects of OPCAB and ONCAB. The study aimed to monitor the hemodynamic parameters after OPCAB and ONCAB and compare the outcome in terms of cardiovascular functioning in first 24 hours after surgery.

## 2. Materials and Methods

This is prospective type of observational analytical study. The study population includes all the patients operated for elective or emergency CABG from January 2015 to June 2016 at Nilratan Sircar Medical College & Hospital. Total 106 patients were studied which includes both OPCAB and ONCAB. Exclusion criteria includes 1) patients with Age < 40 years or >80 years, 2) patients with left ventricular Ejection fraction < 30%, 3) patients with Serum creatinine > 2 mg/dl and 4) patients with Redo operation.

A radial artery line, a central venous catheter and a Swan-Ganz catheter were used as standard hemodynamic monitoring lines. Continuous cardiac output was measured by Edwards Flo Trac device which performs an analysis of the pressure wave signal from the arterial line, correlates the standard deviation of the pulse pressure with the stroke volume using patient demographic data, adjusts for vascular compliance, and then provides continuous cardiac output calculations. The following measurements and calculations were performed: Heart

Rate (HR), Mean Arterial Pressure (MAP), Central Venous Pressure (CVP), Pulmonary Capillary Wedge Pressure (PCWP), Cardiac Output (CO), Cardiac Index (CI), Stroke Volume (SV), Systemic Vascular Resistance Index (SVRI) and Left Ventricular Stroke Work Index (LVSWI). Measurements were made during anesthesia before surgery, postoperatively after 1 and 4 h in the ICU, and in the morning after surgery, approximately after 20 h.

**Statistical Methods**—For comparison between OPCAB and ONCAB groups at different time-points, a two-way ANOVA has been used followed by *post hoc* comparison using Holm-Sidak test when $p$ value is significant. A one-way ANOVA has been done to compare the time-dependent effects of surgery on hemodynamic parameters, followed by *post hoc* comparison using Holm-Sidak test when $p$ value is significant. Comparisons between two groups have been made using unpaired Student's $t$-test. A $p < 0.05$ has been considered statistically significant. All data have been presented as mean ± standard error of mean (SEM).

## 3. Results & Analysis

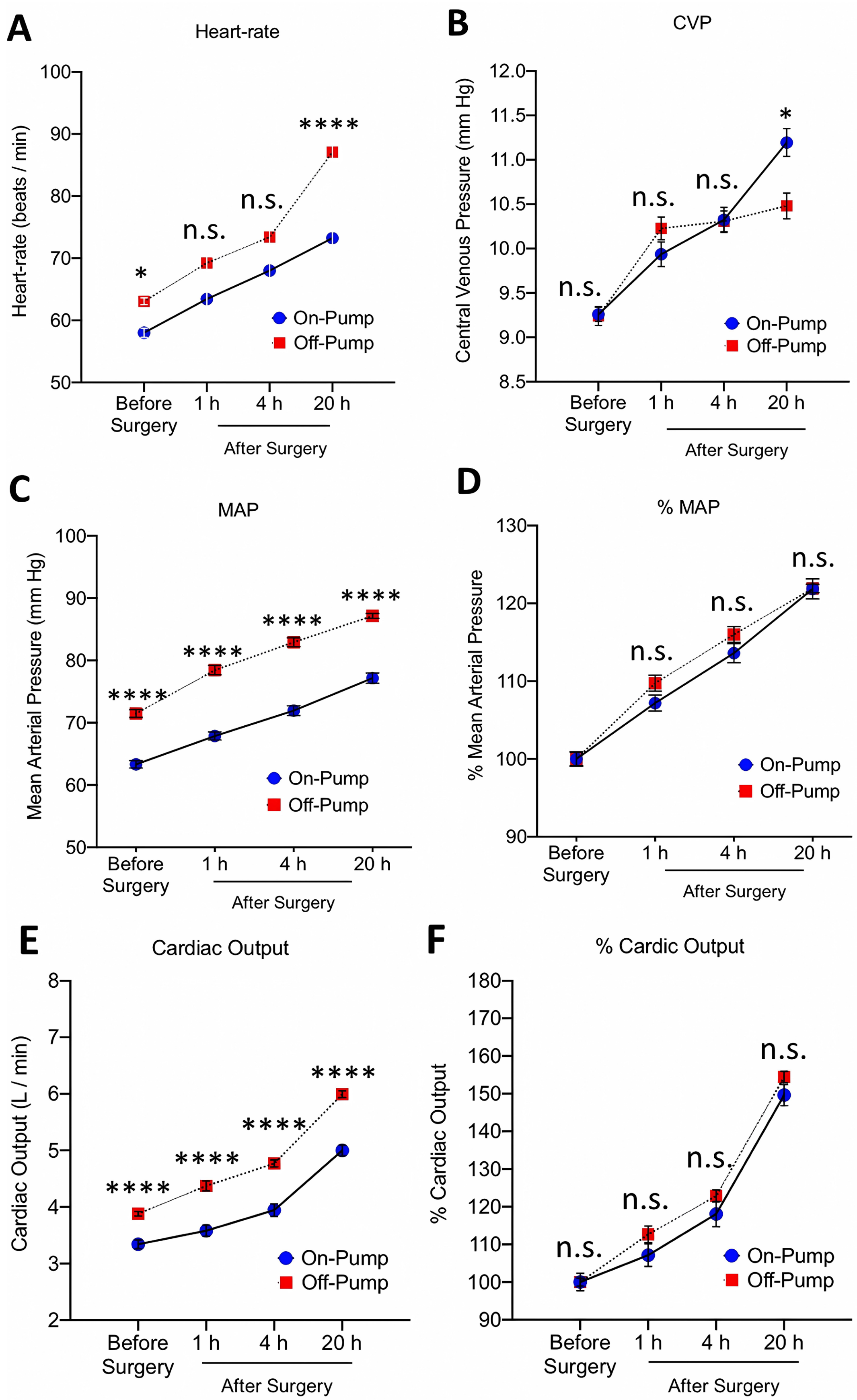
Total 106 patients were operated during the study period with 75 patients being operated for OPCAB and 31 patients being operated for ONCAB. The baseline characteristics (Table 1) of both off-pump and on-pump groups were comparable. The mean age of OPCAB group is 59 years while that of ONCAB group is 57

Table 1. Preoperative baseline characteristics of OPCAB and ONCAB patients.

| PATIENT CHARACTERISTICS | OFF-PUMP CABG (n = 75) | ON-PUMP CABG (n = 31) |
|---|---|---|
| **Mean Age (years)** | 58.87 | 57.19 |
| **Number of male patients (percentage of total)** | 61 (81.33%) | 23 (74.19%) |
| **Number of female patients (percentage of total)** | 14 (18.67%) | 8 (25.81%) |
| **Single vessel disease** | 5 (6.67%) | 0 (0%) |
| **Multiple vessel disease** | 70 (93.33%) | 31 (100%) |
| **Ejection fraction > 50%** | 28 (37.33%) | 8 (25.81%) |
| **Ejection fraction < 50%** | 47 (62.67%) | 23 (74.19%) |
| **Diabetes mellitus** | 22 (29.33%) | 9 (29.03%) |
| **Previous history of myocardial infarction** | 20 (26.67%) | 18 (58.06%) |
| **Peripheral vascular disease** | 3 (4%) | 2 (6.45%) |
| **Chronic obstructive pulmonary disease (COPD)** | 7 (9.33%) | 3 (9.68%) |
| **Treated hypertension** | 39 (52%) | 18 (58.06%) |
| **Treated hypercholesterolemia** | 44 (58.67%) | 17 (54.84%) |
| **Smoking (current or former)** | 45 (60%) | 13 (41.93%) |
| **Average body surface area ($m^2$)** | 1.8 | 1.8 |
| **Average serum creatinine level (mg/dl)** | 0.9 | 0.86 |

years. Majority of the patients of both the groups were male patients. 93 percent patients in OPCAB group had multiple vessel disease and only 5 patients had single vessel disease. On the ONCAB group all the patients had multiple vessel involvement. In OPCAB group 63 percent patient had LVEF (Left Ventricular Ejection Fraction) < 50% and rest have LVEF > 50%. In ONCAB group majority of the patients (74%) had LVEF < 50%. 27% patients in the OPCAB group had previous history of myocardial infarction (MI) while 58% patients in the ONCAB group had previous MI. The prevalence of comorbidities among both the groups was comparable.

Figures 1(A)-(N) demonstrate the Comparison of the effects of OPCAB and ONCAB on the hemodynamic parameters. **(A) HR**—the HR tends to increase slowly in a time dependent manner after both ONCAB and OPCAB. However, in OPCAB group there is significant rise of HR 20 h after surgery. The preoperative baseline HR was little higher in OPCAB group, but it is significantly higher 20 h after surgery in OPCAB group than in ONCAB group. **(B) CVP**—The preoperative baseline CVP before surgery is similar in both the groups. In OPCAB group there is significant rise of CVP at 1 h after surgery and then it remains mostly the same up to 20 h. In ONCAB group there is some rise of CVP at 1 h and 4 h after surgery and significant rise of CVP 20 h after surgery. The absolute value of CVP is significantly higher in ONCAB group at 20 h in comparison to OPCAB group. **(C) MAP & (D) % MAP**—There is steady rise of MAP from the baseline level over the period of observation in both OPCAB and ONCAB group. At each point of observation, the absolute value of MAP is significantly higher in OPCAB group than in ONCAB group. However, the percentage rise of MAP in compared to its baseline level is similar in both the groups over the period of observation. **(E) CO & (F) % CO, (G) CI & (H) % CI**—The preoperative baseline CO (and CI) is higher in OPCAB group than ONCAB group. In OPCAB group there is significant rise of CO (and CI) at 1 h, 4 h and 20 h after surgery. However, in ONCAB group it remains same at 1 h after surgery. There is minimal rise of CO (and CI) at 4 h but significant rise at 20 h after ONCAB. The percentage rise of CO (and CI) in compared to base line level is almost similar in both the groups and the difference is not statistically significant at each time point of observation in between the two groups. **(I) SV & (J) % SV**—There is time dependent rise in SV in both ONCAB and OPCAB groups. The absolute SV is similar in both the groups at 20 h after surgery. However, the pattern of rise is different in the two groups. In OPCAB group there is significant rise of SV at 1 h after surgery and then it remains same. There is further rise of SV at 20 h. In ONCAB group the SV remains almost unchanged in initial hours but significantly rises at 20 h after surgery. The absolute value of SV is significantly higher in OPCAB group at 1 h and 4 h after surgery than in ONCAB group but the difference is eliminated at 20 h. The percentage rise of SV in comparison to its baseline level shows steady rise at 1 h and 20 h after surgery in OPCAB group but the rise at 20 h after surgery is more in ONCAB group although the difference is


A
Heart-rate
Heart-rate (beats / min)
100
90
80
70
60
50
*
n.s.
n.s.
****
On-Pump
Off-Pump
Before Surgery
1 h
4 h
20 h
After Surgery
B
CVP
Central Venous Pressure (mm Hg)
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
n.s.
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On-Pump
Off-Pump
Before Surgery
1 h
4 h
20 h
After Surgery
C
MAP
Mean Arterial Pressure (mm Hg)
100
90
80
70
60
50
****
****
****
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On-Pump
Off-Pump
Before Surgery
1 h
4 h
20 h
After Surgery
D
% MAP
% Mean Arterial Pressure
130
120
110
100
90
n.s.
n.s.
n.s.
On-Pump
Off-Pump
Before Surgery
1 h
4 h
20 h
After Surgery
E
Cardiac Output
Cardiac Output (L / min)
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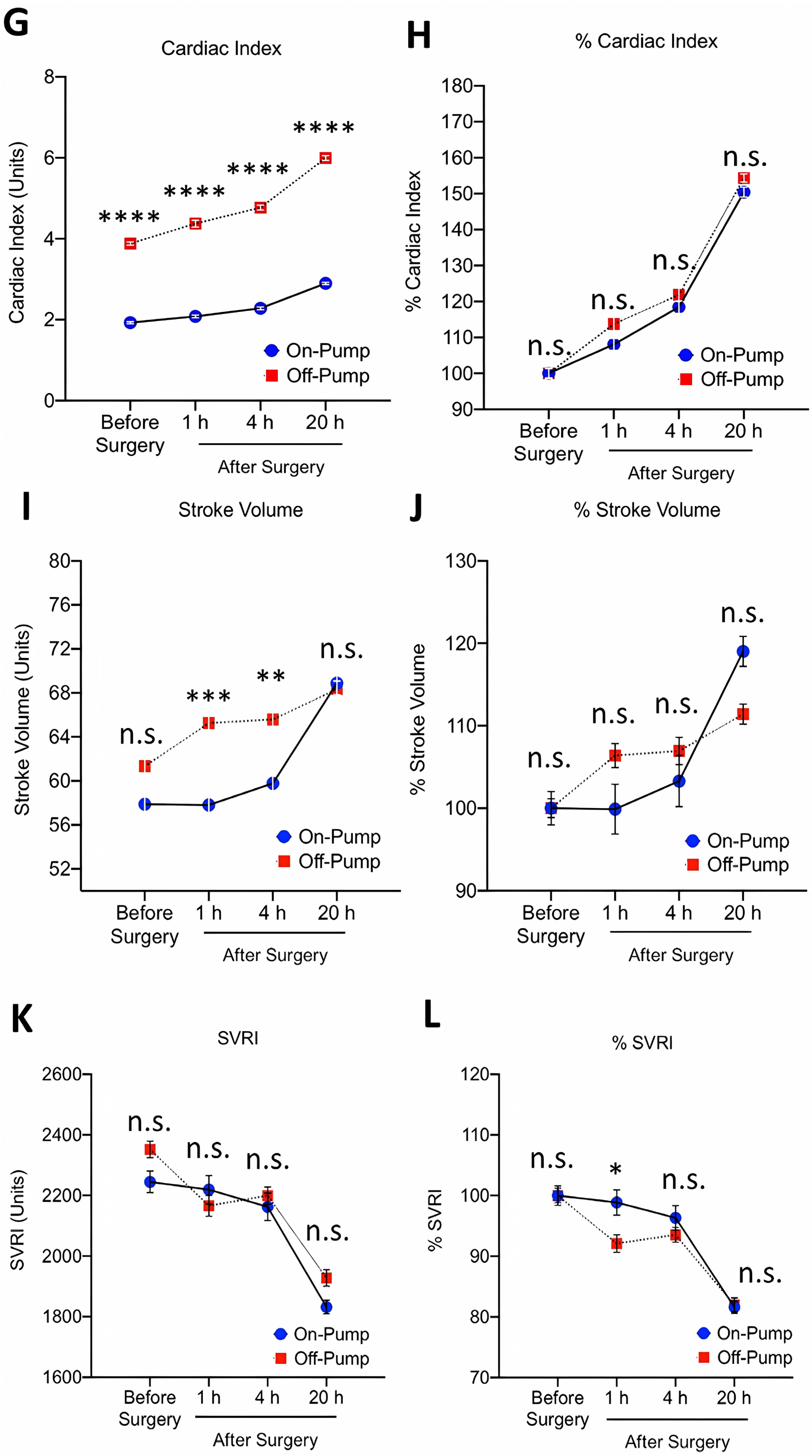
7
6
5
4
3
2
****
****
****
****
On-Pump
Off-Pump
Before Surgery
1 h
4 h
20 h
After Surgery
F
% Cardic Output
% Cardiac Output
180
170
160
150
140
130
120
110
100
90
n.s.
n.s.
n.s.
n.s.
On-Pump
Off-Pump
Before Surgery
1 h
4 h
20 h
After Surgery


G
Cardiac Index
Cardiac Index (Units)
****
****
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On-Pump
Off-Pump
Before Surgery
1 h
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20 h
After Surgery
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% Cardiac Index
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n.s.
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On-Pump
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I
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Stroke Volume (Units)
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SVRI
SVRI (Units)
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% SVRI
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n.s.
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On-Pump
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Before Surgery
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After Surgery

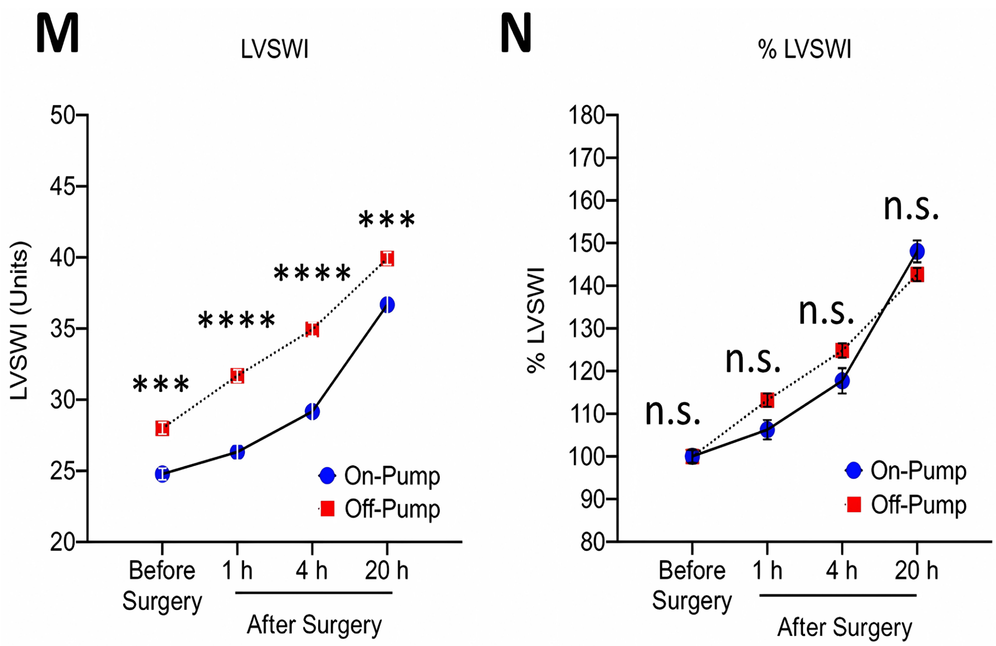


**Figure 1.** (A-N): OPCAB versus ONCAB: Effects on the hemodynamic parameters: (A) Heart-Rate (HR), (B) Central Venous Pressure (CVP), (C) Absolute Mean Arterial Pressure (MAP), (D) Percentage Mean Arterial Pressure (% MAP), (E) Absolute Cardiac Output (CO), (F) Percentage Cardiac Output (% CO), (G) Absolute Cardiac Index (CI), (H) Percentage Cardiac Index (% CI), (I) Absolute Stroke Volume (SV), (J) Percentage Stroke Volume (% SV), (K) Absolute Systemic Vascular Resistance Index (SVRI), (L) Percentage Systemic Vascular Resistance Index (%SVRI), (M) Absolute Left Ventricular Stroke Work Index (LVSWI), (N) Percentage Left Ventricular Stroke Work Index (%LVSWI). [< / > indicates $p < 0.05$, << / >> indicates $p < 0.01$, <<< / >>> indicates $p < 0.001$, <<<< / >>>> indicates $p < 0.0001$ between the groups]; * indicates $p < 0.05$, ** indicates $p < 0.01$, *** indicates $p < 0.001$, **** indicates $p < 0.0001$. "n.s." indicates non-significant difference between the two groups at that particular time-point.

not statistically significant at any point of observation. **(K) SVRI & (L) % SVRI**—SVRI falls significantly after 1 h of OPCAB and then it remains stable and further drops at 20 h. In the ONCAB group there is no significant fall of SVRI in the initial hours. However, it drops significantly at 20 h. The percentage of drop of SVRI in compared to its baseline level is significant in OPCAB group at 1 h after surgery than ONCAB group. **(M) LVSWI & (N) % LVSWI**—There is steady and significant rise of LVSWI in OPCAB group at 1 h, 4 h and 20 h after OPCAB. In ONCAB group, there is some rise of LVSWI at 4 h and significant rise at 20 h. At any point of time the absolute values are significantly high in OPCAB group but while comparing the percentage of increase from base line level, there is no significant difference between the two groups. Table 2 summarizes changes in the hemodynamic parameters in the immediate post-operative period after OPCAB and ONCAB.

## 4. Discussion

The hemodynamic parameters improve in a time-dependent manner after both ONCAB and OPCAB. In the immediate postoperative period the changes of the

**Table 2.** Changes in hemodynamic parameters over time within each group.

| Parameters | Type of surgery | Before surgery (BS) | After surgery (AS) | | | Trends in the alterations within groups |
|---|---|---|---|---|---|---|
| | | | 1 h | 4 h | 20 h | |
| HR (beats/min) | OPCAB | 63.07 ± 0.55 | 69.23 ± 1.17 | 73.41 ± 1.52 | 87.07 ± 1.03 | BS < 1 h = 4 h < 20 h |
| | ONCAB | 58.00 ± 0.73 | 63.42 ± 1.50 | 68.00 ± 2.05 | 73.23 ± 1.02 | BS = 1 h = 4 h = 20 h |
| MAP (mm Hg) | OPCAB | 71.48 ± 8.25 | 78.44 ± 9.06 | 82.92 ± 9.57 | 87.15 ± 10.06 | BS < 1 h < 4 h < 20 h |
| | ONCAB | 63.32 ± 0.59 | 67.87 ± 0.65 | 71.94 ± 0.78 | 77.16 ± 0.81 | BS < 1 h < 4 h < 20 h |
| CVP (mm Hg) | OPCAB | 9.24 ± 0.11 | 10.23 ± 0.13 | 10.31 ± 0.12 | 10.48 ± 0.14 | BS < 1 h = 4 h = 20 h |
| | ONCAB | 9.26 ± 0.08 | 9.94 ± 0.14 | 10.32 ± 0.14 | 11.19 ± 0.16 | BS = 1 h = 4 h < 20 h |
| CO (L/min) | OPCAB | 3.88 ± 0.04 | 4.37 ± 0.08 | 4.77 ± 0.06 | 5.99 ± 0.06 | BS <<<< 1 h <<<< 4 h <<<< 20 h |
| | ONCAB | 3.34 ± 0.08 | 3.58 ± 0.10 | 3.95 ± 0.11 | 5.00 ± 0.09 | BS = 1 h < 4 h <<<< 20 h |
| CI (L/min/m$^2$) | OPCAB | 2.19 ± 0.03 | 2.50 ± 0.04 | 2.68 ± 0.04 | 3.39 ± 0.02 | BS <<<< 1 h <<<< 4 h <<<< 20 h |
| | ONCAB | 1.93 ± 0.02 | 2.08 ± 0.03 | 2.28 ± 0.03 | 2.90 ± 0.03 | BS = 1 h = 4 h <<<< 20 h |
| SV (ml) | OPCAB | 61.33 ± 0.70 | 65.25 ± 0.89 | 65.58 ± 1.01 | 68.33 ± 0.74 | BS < 1 h = 4 h < 20 h (trend) |
| | ONCAB | 57.87 ± 1.17 | 57.81 ± 1.74 | 59.77 ± 1.80 | 68.87 ± 1.05 | BS = 1 h = 4 h <<<< 20 h |
| SVRI (dynes. s/cm$^5$/m$^2$) | OPCAB | 2351.83 ± 27.16 | 2165.72 ± 34.06 | 2199.63 ± 28.59 | 1927.83 ± 27.19 | BS >>>> 1 h = 4 h >>>> 20 h |
| | ONCAB | 2244.58 ± 35.50 | 2219.06 ± 46.76 | 2162.16 ± 45.42 | 1831.61 ± 22.59 | BS = 1 h = 4 h >>>> 20 h |
| LVSWI (g·min/m$^2$) | OPCAB | 27.99 ± 0.42 | 31.68 ± 0.43 | 34.93 ± 0.47 | 39.92 ± 0.43 | BS <<<< 1 h <<<< 4 h <<<< 20 h |
| | ONCAB | 24.77 ± 0.41 | 26.32 ± 0.56 | 29.16 ± 0.73 | 36.68 ± 0.64 | BS = 1 h < 4 h <<<< 20 h |

All values have their respective units (as described in the text). OPCAB: Off-Pump CABG, ONCAB: On-Pump CABG. x < y indicates that the value of the parameter at time "x" is lower (*i.e.* statistically significant) than that at "y". x = y indicates that the value of the parameter at time "x" is similar (*i.e.* statistically non-significant) to that at "y". < / > indicates p < 0.05, << / >> indicates p < 0.01, <<< / >>> indicates $p < 0.001$, <<<< / >>>> indicates $p < 0.0001$. Values represented as mean ± SEM.

hemodynamic parameters are better in OPCAB group than ONCAB group. This may explain the better hemodynamic stability in the initial critical hours after off-pump CABG which potentially benefits further recovery. However, the difference is mostly eliminated after 24 hours and the cardiovascular performances become comparable in both the groups.

The benefit of ONCAB in achieving satisfactory revascularization needs to be titrated against the risk involved due to use of CPB in predicting the outcome and the decision needs to be individualized. The ill effects of CPB are well established in literature and although it can be reduced to great extent by the improvement of modern machines and technologies but it can never be eliminated. The series of inflammatory cascade generated by extracorporeal circulation and cardiac ischemia during cardiopulmonary bypass and cardioplegic arrest can potentially cause multiple organ dysfunction, especially if the organ system is compromised preoperatively. The dysfunction is often temporary and reversible but can have long term sequel [6]. Although there is some degree of systemic inflammatory response after OPCAB as well, but it has been demonstrated in numerous randomized controlled studies that the effect is much less severe than

ONCAB due to avoidance of CPB [7].

In our study, we found that HR tends to rise both after on-pump and off-pump CABG but time dependent rise is more significant after OPCAB than ONCAB group. MAP is higher in OPCAB group before and after surgery but the rate of rise of MAP with time is more or less equal in both the groups. CVP rises significantly at 1 h after OPCAB and 20 h after ONCAB. Vedin, J *et al.* [8] did not find any important difference between on and off-pump group in HR, MAP and CVP.

While comparing the CO and CI between off-pump and on-pump group we found that CO and CI is higher in OPCAB group than ONCAB group at any point of time before surgery or after surgery (1 h, 4 h or 20 h). This may be because of non-randomization of the study population and because of the fact that a good number of patients with low cardiac output (low LVEF) or with hemodynamic instability has been selected for ONCAB. But while comparing the time dependent rise of CO and CI post-operatively in respect to preoperative status, we found that CO and CI rise significantly in a time-dependent manner up to 20 h in the off-pump group (BS << 1 h << 4 h << 20 h) but in on-pump group the CO and CI rises slowly in the immediate post-operative period at 1 h and 4 h, but at 20 h it rises significantly (BS = 1 h < 4 h << 20 h). However, in the percentage chart the difference is eliminated at 20 h. The immediate improvement of CO and CI after OPCAB may be due to increased myocardial revascularization immediately after surgery through the grafts bypassing the occluded coronary arteries. In the ONCAB group this defect may be masked due to the immediate effect of CPB and cardioplegia. Vedin, J *et al.* [8] also found CO and CI to increase significantly over time in first 24 hours after OPCAB.

Increased myocardial contractility after OPCAB was also suggested by improving stroke volume in a time dependent manner when compared with baseline value before surgery. SV does not increase significantly in initial hours after ONCAB but it increased significantly at 20 h. In OPCAB group the increase in SV is significant in each observation in the immediate post-operative period. Vedin, J *et al.* [8] showed that the decrease in SV between the measurements at baseline and 1 h ($p = 0.01$) is less after OPCAB than after ONCAB. Similar tendency was noticed at 4 h (p = 0.07) although there is no significant difference in measurements at 20 h ($p > 0.2$) in between the two groups.

SVRI decreases significantly from its pre-operative value at 1 h post-operatively in the OPCAB group. In contrast, SVRI does not change significantly in the immediate post-operative hours in ONCAB group. This is due to better peripheral vasodilatation in the off-pump patients in the immediate post-operative hours in comparison to on-pump group. Vedin, J *et al.* [8] also found significantly reduced SVRI at 1 h post-operatively in the off-pump group than on-pump patients.

LVSWI was increased significantly in a time dependent manner after OPCAB in our study. The result is as per the findings of Vedin, J *et al.* [8]. This is due to better myocardial contractility after revascularization. In ONCAB group, the time dependent rise is significantly low in the immediate post-operative hours although the difference is eliminated at 20 h. This can explain the less common occurrence of low cardiac output syndrome in the immediate postoperative period after OPCAB than ONCAB as mentioned in other studies [9] and justifies the safe use of OPCAB in patients with left ventricular dysfunction whenever feasible [10]. Although Louagie *et al.* [11] mentioned no significant difference among the hemodynamic parameters (CI, LVSWI and SVRI) in between ONCAB and OPCAB, but there is higher requirement of inotropic supports in ONCAB patients in compared to OPCAB patients.

Numerous factors can potentially induce a low cardiac output state after ONCAB. CPB itself causes myocardial dysfunction by multiple inflammatory mediators like cytotoxins, activated neutrophils, monocytes and micro emboli and regional ischemia. Aortic cross-clamping and cardioplegia invariably produce myocardial edema and flaccid distension which further reduces myocardial contractility. In an already compromised myocardium, the weaning of CPB may be complicated by reperfusion injury after ischemia as well as other factors like preload afterload mismatch that increases left ventricular end-diastolic volume, myocardial wall stress and oxygen demand [12] [13]. Arrhythmia, especially Atrial Fibrillation (AF), which is seen in 20% - 40% patients after CABG [14], is another factor that can contribute to low cardiac output state after CABG. Ascione *et al.* in their large RCT has showed that CPB with cardioplegia is an independent predictor for AF after CABG [15]. The patients who have a preexisting identifiable electrophysiologic substrate are at increased risk of developing AF after CPB [16]. On the other hand, the incidence of AF is much lower after OPCAB as demonstrated in four meta-analyses [16] [17] [18] [19]. The pooled analysis of the Beating Heart Against Cardioplegic Arrest Studies (BHACAS 1 and 2) done by Angelini *et al.* showed the significant decrease in the incidence of AF from 37% to 13% with OPCAB [20].

Perioperative myocardial infarction (MI) is another important factor that profoundly affects the hemodynamic parameters and induces a low cardiac output state after CABG. The incidence of MI is found to be comparable after OPCAB and ONCAB in multiple studies. However, the recovery of myocardial oxidative metabolism is much faster after OPCAB. This is supported by lower requirement of inotropic supports due to better contractility after OPCAB. Selvanayagam and colleagues assessed the degree of irreversible myocardial damage and global left ventricular dysfunction after CABG by cardiovascular magnetic resonance imaging (MRI). They found that the left ventricular function in the immediate post-operative period is much better after OPCAB than ONCAB ($p$ = 0.04) but the there is no significant difference in incidence and extent of irreversible myocardial injury [21].

To summarize, our study demonstrates the better cardiovascular function in the immediate post-operative period after OPCAB than ONCAB. This is explained by the avoidance of CPB and cardioplegic arrest which significantly reduces the risk of global myocardial ischemia and myocardial stunning.

## Limitation of the Study

The study is not completely free from selection bias and the patients were not distributed randomly in both the groups. All the patients with single vessel disease were operated by OPCAB, while all the patients in ONCAB group have significant multi-vessel disease. The fraction of patients having previous myocardial infarction and preoperative poor left ventricular function is higher in ONCAB group in compare to OPCAB group.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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