

Assessment of Right Ventricular Function in Patients undergoing Coronary Artery Bypass Graft : a single center study

Salma Taha (✉ esmaeil.salma@gmail.com)

Assiut University

Ahmed Taha

Assiut University

Maria Refaat

Assiut University

Hussein ElKhayat

Assiut University

Salah Atta

Assiut University

Ahmed Mandour

Assiut University

Ahmed Abdel-Galeel

Assiut University

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Abstract

Background:

Right ventricular dysfunction (RVD) is a major risk factor in coronary artery disease (CAD). In patients undergoing revascularization for left ventricular ischaemia, the incidence of RVD is reported in about 20%.

Aim:

this study aimed to assess right ventricular function as a possible risk factor in patients undergoing coronary artery bypass graft.

Patients and methods:

This prospective study was conducted on 77 patients underwent coronary artery bypass grafting. All cases were subjected to detailed medical history, full physical examination, electrocardiogram, routine laboratory tests including echocardiography or STE, all parameters obtained before, within 1 week and 6 months after surgery.

Result:

Though, LVEF preoperatively was insignificantly higher in RVD group compared to non RVD ($P=0.84$), LVEF 1 week postoperatively was significantly lower in RVD group compared to non RVD group ($p=0.03$) at the same time there was significant reduction in LVEF in both groups 1 week postoperatively compared to preoperatively and this was more obvious in RVD group compared to non RVD group ($p=0.001$). In addition, the diastolic function (E/A ratio) preoperatively was significantly lower in RVD group compared to non RVD group ($p=0.03$). Moreover, the mean longitudinal strain was significantly higher in non RVD group compared to RVD group preoperatively ($p=0.012$).

Conclusion:

It is suggested that at the early days after the CABG surgery there is a decline in right ventricle function which is relatively reversible at longer intervals (6 months after surgery). Hence, it is recommended to conduct longer studies to evaluate right ventricular function after CABG surgery.

Introduction

Right ventricular dysfunction (RVD) is a major risk factor in coronary artery disease (CAD) patients and approximately 20% of CAD patients undergoing revascularization for coronary artery ischaemia have this combination [1]. RVD is a possible cause of cardiac failure after cardiac surgery and has a high mortality rate [2, 3]. RVD is a recognized cause of hypotension early after coronary artery bypass graft surgery (CABG) [3–5].

A decrease in RV function is an event known to occur after CABG. RVD can be seen during and immediately after cardiac surgery. Although the mechanism of this phenomenon is not well understood. Cardiopulmonary bypass, perioperative myocardial ischemia, myocardial damage during operation, prolonged cardioplegia, and pericardial disruption or adhesion have been suggested as probable causes. [4–6]

The decision for CABG surgery is related to multiple factors mainly degree of angina, function of left ventricle (LV), burden of ischemia, and anatomy of coronaries [7]. Major reasons for complications of cardiac surgery are the need for hypothermic cardiac arrest, aortic cross clamping, and exposure to a cardiopulmonary bypass (CPB) [8, 9]. It has been postulated that avoidance of these factors by performing off-pump coronary artery bypass (OPCAB) surgery might reduce perioperative morbidity and improve outcome [8]. The portion of CABG on the beating heart without the use of CPB has been expanded in cardiac surgery as a result of awareness of the damaging effect of CPB [9, 10].

A few studies focused on hemodynamic alternations associated with OPCAB reported that reduced functions of both ventricles during coronary artery anastomosis are the main mechanism of hemodynamic derangements and especially, impaired diastolic function of the right ventricle (RV) plays an important role [12–14]. It is reported that the major cause of hemodynamic changes during OPCAB was disturbed diastolic filling of the RV through the measurement of chamber pressures or monitoring of echocardiography [13]. However, clinical studies evaluating the change in RV function in patients with ischemic heart disease are very rare.

It was noted that, there is no significant change in the RV ejection fraction (RVEF) and cardiac index during anastomosis of the left anterior descending artery and right coronary artery. However, reduced RVEF accompanied by an increase in RV afterload and decrease in the cardiac output (COP) was observed during anastomosis of the obtuse marginal (OM) artery. The displacement of beating heart for positioning during anastomosis of the graft to OM artery causes significant derangement of RV function and decrease in COP [15].

This study aimed to evaluate the effect of CABG either ONCAB or OPCAB on the RV function using conventional echocardiography and speckle tracking echocardiography. To our knowledge, it is the first study to assess RV function using STE prior to CABG surgery.

Patients and methods

This prospective study was conducted in Assiut University Heart Hospital from May 2019 till April 2021. Sample size calculation was conducted using G*Power 3 software. A calculated minimum sample of 70 patients was needed to detect an effect size of 0.25² in the variance of RVD on three points of time (baseline, 1-week, and 6-months) repeated measurements with 0.5 correlation among repeated measurements and 1 no sphericity correction, with an error probability of 0.05 and 80% power.

Patients underwent elective CABG of different age groups and of both sexes were included while those with poor echo window, prior RV dysfunction and with LV dysfunction (EF < 40%) or refused to participate were excluded. All patients were subjected to the followings:

- **Clinical evaluation;** including thorough history taking focusing on socio-demographics such as age and sex. Other relevant clinical data such as smoking, dyslipidemia and previous myocardial infarction (MI) was reported. Full physical examination was conducted.

- **12 leads ECG.**

- **Routine laboratory tests** including renal functions.

- **Echocardiography** either conventional echocardiography or STE.

Conventional echocardiography:

All enrolled patients had transthoracic echocardiography (according to the ASE guidelines)[16] which was done preoperative, early post-operative (within one week) and 6 months postoperative by the same operator and the same machine (Phillips healthcare Epic 7 C, release 1.7.1) using S5-1 probe for 2D data. TAPSE was measured using M mode at lateral tricuspid annulus in apical 4 chamber view. Modified apical 4 chamber view focused on RV used to measure RV area by tracing the RV endocardium during systole and diastole to calculate RVFAC. Pulsed wave Doppler used to measure RVS´ in subcostal 4 chamber view by placing TDI sample volume at lateral tricuspid annulus. RSVS measured by calculated volumes (end systolic volume (ESV) and end diastolic volume (EDV)) and heart rate (HR).

Speckle tracking echocardiography:

Apical four-chamber views were specifically optimized to visualize the right ventricle and obtain echocardiographic cine loops by recording three consecutive heart cycles (>61 frames per second). Data were stored in DICOM format, and offline analyses were performed using QLAB 10.4 software (Philips Healthcare). After entering the aCMQ interface to determine the apical four-chamber view and choosing the AP4 option, a region of interest was traced by tracing the endocardial border on an end-diastolic frame by clicking three separate points (apex, lateral, and septal points of the tricuspid annulus) end-diastole in the right ventricle from the RV-focused view. The region of interest (ROI) was automatically estimated and adjusted to fit the thickness of the RV free wall and the septum. Adequate tracking was verified in real time and was corrected by adjusting the ROI or manually correcting the contour to ensure optimal tracking. Special care was taken to fine-tune the region of interest using visual assessment during cine loop playback, to ensure that the segments were tracked appropriately.

- **CABG Surgery:** All study patients underwent CABG surgery either ONCAB or OPCAB according to patients' clinical data and surgeon preference.

Statistical analysis:

Data were verified, coded by the researcher, and analyzed using IBM-SPSS 24.0 (IBM-SPSS Inc., Chicago, IL, USA)*. Descriptive statistics: Means, standard deviations, medians, ranges, frequency, and percentages were calculated. Test of significances: Chi square/Fisher's exact/Monte Carlo Exact (MCE) test was used to compare the difference in distribution of frequencies among different groups. For normality testing, a Kolmogorov-Smirnov test indicated that the main continuous variables under study followed a normal distribution, $D(77) = 0.168-0.071$, $p = 0.159-0.201$. Student t-test analysis was carried out to compare the means of dichotomous data. Paired Sample t-test analysis was carried out to compare the means of dichotomous data on repeated measure. The clinical and demographic factors with proven statistical significance from the univariate analyses were further included in the multivariate logistic regression models for prediction of RVD at the 1st postoperative week. McNemar's test was used to determine if there is difference on the frequency of RVD after one-week and after six months postoperatively. Significant p value was considered when it is <0.05 .

Ethical Consideration:

IRB approval was obtained from the Medical Ethic Committee, Faculty of Medicine, Assiut University. Trial registration was prospectively undertaken in clinical trial.gov (NCT03275220)(07/09/2019). The study was carried out in accordance with the Helsinki Declaration guidelines.

A written informed consent was obtained from the patient before the participation in the study. All collected data was confidential and was used for the purpose of scientific research only. Every research participant had the complete right and freedom to withdraw at any time from the study without any consequences on the medical service provided.

Results

118 patients underwent elective CABG of different age groups and of both sexes at Assiut University Heart Hospital, during the study period. Only 77 patients (77% males; mean age \pm SD 56 ± 9.3 years (range 30-75) were enrolled and consented the study participation, Fig.1.

Table (1) summarizes the clinical, echocardiographic, laboratory and surgical criteria of the study population.

Furthermore, the outcome of the procedure at 1-week postoperatively showed that 43 patients had normal RV function, 17 patients had RVD, 9 patients died, and 8 patients had bad echo window on follow up **(Table 1)**.

Regarding the risk factors for ischemic heart disease (IHD), 29 patients had hypertension, 32 were diabetic, 31 were smokers and. Only 9 patients reported history of MI and 5 patients previously had PCI **(Table 2)**.

In this study, 11 patients of the studied group had LIMA graft to LAD artery, 30 had both LIMA graft to LAD artery and SVG to OM artery and 19 had LIMA graft to LAD artery and SVG to RCA artery (**Table 3**).

By conventional echocardiography LVEF preoperatively was insignificantly higher in RVD group compared to non RVD ($P = 0.842$) while LVEF at 1-week postoperatively was significantly lower in RVD group compared to non RVD group ($p = 0.032$) at the same time there was significant reduction in LVEF in both groups at 1-week postoperatively compared to preoperatively and this was more obvious in RVD group compared to non RVD group ($p = 0.001$).

Also, TAPSE preoperatively was insignificantly lower in RVD group than non RVD group ($p = 0.071$) while at 1-week postoperatively TAPSE was significantly lower in RVD group than non RVD group ($p = 0.012$). There was significant reduction in TAPSE in both groups 1 week postoperatively compared to preoperatively and this was more obvious in RVD group compared to non RVD group ($P = 0.013$).

Moreover, FAC preoperatively was significantly lower in RVD group than non RVD group ($p = 0.008$) also at 1-week postoperatively FAC was significantly lower in RVD group than non RVD group ($p = 0.041$). There was significant reduction in FAC in both groups 1 week postoperatively compared to preoperatively ($P = 0.001$).

As regards the TDIS velocity there was no significant difference between both groups preoperatively ($p = 0.422$) on the other hand TDIS velocity at 1-week postoperatively was significantly lower in RVD group than non RVD group ($p = 0.001$). There was significant reduction in TDIS velocity in both groups at 1-week postoperatively compared to preoperatively and this was more obvious in RVD group compared to non RVD group ($p = 0.001$).

In addition to this the diastolic function (E/A ratio) preoperatively was significantly lower in RVD group compared to non RVD group ($p = 0.03$), but E/A ratio at 1-week postoperatively was insignificantly lower in RVD patients than non RVD patients ($p = 0.7$), so there was significant improvement in diastolic function in RVD group at 1-week postoperatively compared to preoperatively ($p = 0.04$) (**Table 4**).

Using STE, the mean longitudinal strain (LS) was significantly higher in non RVD group compared to RVD group preoperatively ($p = 0.012$), also, the mean LS was significantly higher in non RVD patients than RVD patients at 1-week postoperatively ($p = 0.001$), but there was significant reduction in mean LS in both groups at 1-week postoperatively compared to preoperatively and this was more obvious in RVD group ($p = 0.001$) (**Table 4**).

Among the studied samples ($n = 77$), 68 cases were followed up at 6-months, they were divided into 60 cases who did not have RVD and eight patients with RVD, among those with RVD, four cases developed RVD at 6-months postoperatively and half of the cases persisted to have RVD. On the other hand, 13 cases with RVD at 1-week were improved at 6-months and this was statistically insignificant ($P = 0.153$). Male patients insignificantly represented 87% of RVD group and 78% of non RVD group ($P = 0.521$). Also, diabetic patients represented insignificantly higher percentage of RVD patients compared to non RVD

patients ($p = 0.334$). Moreover, hypertensive patients represented higher percentage of non RVD patients compared to patients with RVD, and this was statistically nonsignificant ($p = 0.711$). For smoking as a risk factor, smokers represented a higher percentage of RVD group compared to non RVD patients and this was statistically significant ($p = 0.042$).

As regard levosimendan as a positive inotropic drug administered intraoperatively, the percentage of patients needed Levosimendan was higher in RVD group than non RVD group. Also, seven cases out of 13 who had RVD on 1-week and improved on 6-months had received intraoperative levosimendan and this was statistically insignificant ($p = 0.621$) as shown in **table 5**.

Discussion

The main findings of the current study were that about one-third of patients who underwent CABG developed RV dysfunction 1-week postoperatively and 75% of which improved at 6-months follow up. Also, being male with hypertension, smoking and low LVEF at 1-week postoperatively were the main independent predictors of RV dysfunction post-CABG.

In this study, male represented 77%, age ranged from 30 to 75 years with mean 56 ± 9.3 years. In agreement with this among 50 patients included in the Korshin, et al, study, the gender distribution revealed more male than female patients [17]. Also, for multivariate logistic regression model as a predictor of high RVD, the final model showed that hypertensive patients had eight times more risk of RVD compared to non-hypertensive patients ($P = 0.032$) and this was in agreement with Varma, et al., study in which the patients at the time of CABG surgery showed that hypertension was the commonest risk factor [18].

For smoking as a risk factor, smokers significantly represented about double the percentage in RVD group compared to non RVD patients ($p = 0.013$), and this was in agreement with the study of Hammal F et al., Who found that there was increased RVD with smoking and that patients who continued to smoke had significantly lower survival rates compared with quitters in CABG group [19].

Further, in this study, 11 patients of the studied group had LIMA graft to LAD artery, 30 had both LIMA graft to LAD artery and SVG to OM artery and 19 had LIMA graft to LAD artery and SVG to RCA artery. Similarly, in study by Gozdzik, et al., 69 patients undergoing elective CABG were included and in all LIMA graft to the LAD artery was used [20].

Moreover, in the current study, 41 patients had significant RCA stenosis and only 25 of them had RCA graft, the reason for not grafting other RCAs was that either the vessel was too small/diffusely diseased or that it was non dominant. RCA lesion insignificantly represented higher percentage of RVD than non RVD groups. This was in accordance with Pegg, Selvanayagam [21] who studied the effect of off-pump versus on-pump coronary artery bypass grafting on early and late RV function and found that there was relative reduction in RV function early postoperatively and despite the high percentage of RCA stenosis,

there remained no difference in any parameter of RV function between those receiving a graft and those who did not [21].

The results of this work showed that there was 50% insignificantly increased need to use intraoperative Levosimendan in patients with RVD ($P = 0.481$), also that 7 cases out of 13 who had RVD on 1-week postoperative follow up and improved on 6 months follow up had received intraoperative levosimendan and this was statistically insignificant ($p = 0.601$). This was in agreement of the result of Toller, Heringlake [22] Who found that levosimendan effectively improves general and pulmonary hemodynamics in patients undergoing cardiac surgery, thereby reducing the need for inotropic agents and mechanical circulatory support, and additionally optimizing renal and hepatic function. In general, the length of stay on the ICU and in the hospital is shortened. Overall, levosimendan treatment is considered as a kind of “safety net” in the surgical setting. The unique inotropic and cardio protective properties of levosimendan can provide sustained effects for several days and can thus help to reduce complications in the postoperative period [22]

Additionally, by conventional echocardiography, there was statistically significant decrease in FAC, TAPSE and RVS` in the group with RV dysfunction ($p < 0.05$). On the other hand, diastolic function (E/A ratio) preoperatively was significantly lower in RVD group compared to non RVD group ($p = 0.031$), but at 1-week postoperatively there was significant improvement in diastolic function in RVD group compared to preoperatively ($p = 0.042$).

It is still unclear to detect the possible reason of deformation of RV geometry, reduction of TAPSE and RVS' after open heart surgery. Many factors may contribute to such as changed contraction pattern of the interventricular septum, pericardiotomy mechanical effects, or postoperative adhesions of the RV. Many theories were proposed for explanation of this dysfunction as pericardial opening, injury to the right atrium during cannulation, incomplete myocardial protection, and adhesions between the RV and nearby mediastinal structures [23, 24], RVEF is unchanged and cardiac output is not reduced [25]. This agreed with the study of Ordiené R et al., who studied the changes of biventricular function after CABG surgery and found significant early postoperative deterioration in TAPSE, RV S' and FAC [26]. Also, in agreement with this study, Korshin, et al., found significant reduction in TAPSE after surgery [16]. Another study by Rösner, et al., indicated that post CABG tricuspid TAPSE was markedly reduced, but there was unchanged RVFAC.[25]

Likewise, among 46 patients who were eligible for CABG and included in a study by Hashemi, et al., on impact of CABG on RV function, TAPSE reduced substantially after CABG ($p < 0.001$), with significant decline in RVS` that was also observed following CABG ($p < 0.001$) [27]. In contrast to this study, Larrazet, et al., found that RVFAC remained unchanged despite there was a decline in RVSV after CABG [28]. Likely, SHI et al., studied RV diastolic dysfunction after CABG and found that right ventricular diastolic performance was impaired early after CABG surgery [29].

Regarding the STE, the mean LS was significantly lower in RVD group compared to non RVD group preoperatively ($p = 0.012$), also, the mean LS was significantly lower in RVD patients than non RVD

patients 1-week postoperatively ($p = 0.001$), but there was significant reduction in the mean LS in both groups at 1-week compared to preoperative mean and this was more obvious in RVD group ($p = 0.001$). In concordance with the result of our study, Rong et al., studied RV function after CABG by 2D speckle tracking and found that there was significant reduction in RVD group postoperatively [30]. Similarly, Gozdzik, et al., enrolled 69 patients scheduled for CABG; they observed a significant decrease in the RVGLS post operatively [20]. As well, among 24 patients undergoing elective CABG enrolled by Bitcon, et al., there was a significant decrease in RV free wall strain [31].

In our study, among the studied samples ($n = 77$), 68 cases were followed up at 6 months, they were divided into 60 cases did not have RVD and 8 patients who had RVD, among those who had RVD, 4 cases developed RVD at 6 months postoperative follow up and half of the cases persisted to have RVD. On the other hand, about 13 cases who had RVD at 1 week postoperative follow up were improved at 6 months follow up which was statistically insignificant ($P = 0.15$). This was similar to Pegg, Selvanayagam [21] who studied effects of off-pump versus on-pump coronary artery bypass grafting on early and late right ventricular function and found that the early reduction in measures of RV function recovered completely by 6 months, with normalization of all volumetric parameters [21].

Furthermore the study of Ordiené, Unikas [32] who studied the changes of biventricular function after CABG surgery and found that the reduction was seen right after the surgery had a tendency to improve within the follow-up period (6 months) [32]. Similarly, the study of John, Thomas [33] found significant increase in the RV function post CABG over the next 2 months after surgery [33]. This study was against the study of Chinikar, Rafiee [34] who studied RV dysfunction correlates in patients after CABG and found that RVD remains till 6-months postoperative follow up [34].

Conclusion and Recommendations

In conclusion, this study revealed high prevalence of most of the cardiovascular risk factors especially smoking, hypertension and male sex in patients underwent CABG. Also, it was found that at the early days after the CABG surgery there was a decline in the RV function which is relatively reversible at longer intervals (6-months after surgery). It is recommended to conduct longer studies to evaluate right ventricular function after CABG surgery. Also, a large-scaled multi-center study may be more informative. As well, using cardiac MRI parameters as CMR is ideally suited for assessment of the RV because it allows comprehensive assessment of cardiovascular morphology and physiology without most of the limitations that hinder alternative imaging modalities.

Limitations

The current study encountered several limitations; it was single-center study, this may endanger the generalizability of the study. Assessment of 2D speckle tracking is currently available only in the apical four chamber view whereas the assessment of the LV strain is obtained by the three classic views. The lack of other independent diagnostic modalities as magnetic resonance imaging.

Declarations

Ethics approval: IRB approval was obtained from the Medical Ethic Committee, Faculty of Medicine, Assiut University. Trial registration was prospectively undertaken in clinical trial.gov (NCT03275220) (07/09/2019). The study was carried out in accordance with the Helsinki Declaration guidelines. A written informed consent was obtained from the patient before the participation in the study. All collected data was confidential and was used for the purpose of scientific research only. Every research participant had the complete right and freedom to withdraw at any time from the study without any consequences on the medical service provided.

Consent for publications: NA

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Tables

Table 1: Demographic characteristics of the studied sample

Variable	Category	n = 77
Age in years	• Mean ± SD	56.01 ± 9.3
	• Median (Range)	56 (30 – 75)
Sex	• Male	59 (76.6%)
	• Female	18 (23.4%)
Operative Technique	• ONCABG	52 (67.5%)
	• OPCABG	25 (32.5%)
Outcome at 1-week PO	• Normal	43 (55.8%)
	• RVD	17 (22.1%)
	• Died	9 (11.7%)
	• Bad Echo	8 (10.4%)

Table 2: Relationship between Baseline Characteristics and RVD at 1-week FU

	No-RVD (n= 43)	RVD (n= 17)	P-value
DM			= 0.540*
• No	19 (44.2%)	9 (52.9%)	
• Yes	24 (55.8%)	8 (47.1%)	
HTN			
• No	21 (48.8%)	10 (58.8%)	= 0.485*
• Yes	22 (51.2%)	7 (41.2%)	
Smoking Status			= 0.013**
• No	14 (32.6%)	2 (11.7%)	
• Smoker	17 (39.5%)	14 (82.4%)	
• Ex-smoker	12 (27.9%)	1 (5.9%)	
Previous Cardiac Event			
• MI	5 (11.6%)	4 (23.5%)	= 0.043***
• PCI	5 (11.6%)	0 (0%)	= 0.041***

*Chi-square test was used to compare differences in frequency between groups.

**MCE test was used to compare differences in frequency between groups.

***Fisher's Exact test was used to compare differences in frequency between groups.

Table 3: Cardiac-related Characteristics of the studied groups

	No-RVD (n= 43)	RVD (n= 17)	P-value
Graft Type			= 0.309**
• LIMA to LAD	8 (18.6%)	3 (17.6%)	
• LIMA to LAD, SVG to OM	23 (53.5%)	7 (41.2%)	
• LIMA to LAD, SVG to RCA	12 (27.9%)	7 (41.2%)	

*Chi-square test was used to compare differences in frequency between groups.

Table 4: Comparison of Echo Parameters between the studied groups

(Mean ± SD)	No-RVD (n= 43)	RVD (n= 17)	P-value*
LVEF%			
• Pre-	56.93 ± 6.8	57.35 ± 7.5	= 0.835
• 1-Week PO	55.02 ± 5.3	51.82 ± 5.1	= 0.033
P-value**	< 0.001	< 0.001	
TAPSE			
• Pre-	21.77 ± 3.1	19.23 ± 3.0	= 0.067
• 1-Week PO	17.53 ± 3.3	12.79 ± 2.8	= 0.010
P-value**	< 0.001	= 0.011	
FAC%			
• Pre-	45.94 ± 9.9	38.24 ± 9.2	= 0.008
• 1-Week PO	38.26 ± 8.3	32.65 ± 5.1	= 0.036
P-value**	< 0.001	= 0.030	
TDIS			
• Pre-	11.94 ± 2.4	11.38 ± 2.4	= 0.427
• 1-Week PO	10.39 ± 1.8	8.47 ± 1.6	= 0.001
P-value**	< 0.001	= 0.001	
E/A Ratio			
• Pre-	0.89 ± 0.2	0.76 ± 0.1	= 0.030
• 1-Week PO	0.92 ± 0.2	0.90 ± 0.3	= 0.724
P-value**	= 0.456	= 0.048	
LS			
• Pre-	-26.02 ± 2.1	-22.65 ± 2.1	= 0.012
	-23.47 ± 3.2	-16.94 ± 2.1	< 0.001

• 1-Week PO		
P-value**	= 0.013	< 0.001

*Independent t-test was used to compare the mean differences.

**Paired Sample t-test was used to compare the mean differences.

Table 5: RVD among the studied sample at 6 month follow up (n=68):

	RVD	No RVD	P-value*
Total patients at end of the study	8 (11.8%)	60 (88.2%)	
Newly developed RVD at 6-month	4 (5.9%)	64 (94.1%)	
Levosimendan	5 (62.5%)	28 (46.7%)	= 0.623
RV dysfunction at 1-week	4 (50%)	13 (25.5%)	= 0.152
Male gender	7 (87.5%)	47 (78.3%)	= 0.541
DM	6 (75%)	31 (51.7%)	= 0.273
HTN	3 (37.5%)	29 (48.3%)	= 0.712
Smoking	5 (62.5%)	30 (50%)	= 0.041

*Chi-square test was used to compare differences in frequency between groups.

Figures

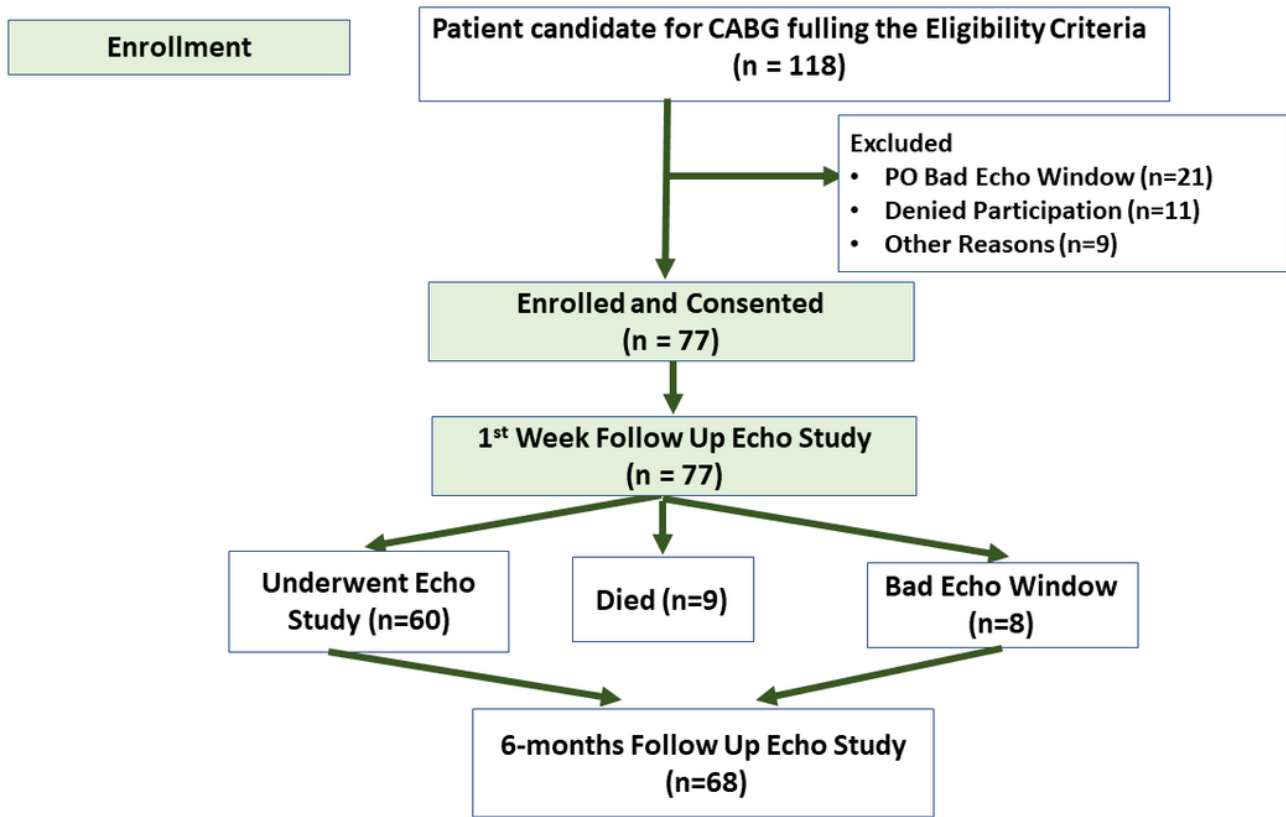


Figure 1

Flow Chart Diagram for the Study Participants