Journal of Visualized Experiments

Noninvasive determination of vortex formation time using transesophageal echocardiography during cardiac surgery

--Manuscript Draft--

Article Type:	Invited Methods Article - JoVE Produced Video
Manuscript Number:	JoVE58374R1
Full Title:	Noninvasive determination of vortex formation time using transesophageal echocardiography during cardiac surgery
Keywords:	Transmitral blood flow efficiency; vortex formation time; early left ventricular filling; Fluid Mechanics; diastolic function; intraventricular blood flow; continuity equation; transesophageal echocardiography
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Additional Information:	
Question	**Response**
Please indicate whether this article will be Standard Access or Open Access.	Standard Access (US$2,400)
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August 3, 2018

Alisha DSouza PhD
Senior Review Editor
Journal of Visualized Experiments

RE: Revision of JoVE58374 "Noninvasive determination of vortex formation time using transesophageal echocardiography during cardiac surgery" by PS Pagel et al

Dear Dr. DSouza:

Thank you for the critical reviews of our manuscript. I revised our manuscript in response to your comments and those of the referees. As can be seen in my responses to the comments, I incorporated all of the changes into the revised manuscript. I verified that the .tif images comply with the journal requirements that you emphasized in your letter of June 27. Please note that I do not use the "track changes" function of Microsoft Word; I find this software cumbersome and difficult to follow. As a result of your efforts, I believe that the manuscript has been substantially improved as a result, and I hope that you will now find it acceptable for publication in the *Journal of Visualized Experiments.*

Thank you for your consideration, and best wishes.

Sincerely,

Paul S. Pagel MD PhD

TITLE:

Noninvasive Determination of Vortex Formation Time using Transesophageal Echocardiography During Cardiac Surgery

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KEYWORDS:

Trans-mitral blood flow efficiency, vortex formation time, early left ventricular filling, fluid mechanics, diastolic function, intraventricular blood flow, continuity equation, transesophageal echocardiography

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SUMMARY:

We describe a protocol to measure vortex formation time, an index of left ventricular filling efficiency, using standard transesophageal echocardiography techniques in patients undergoing cardiac surgery. We apply this technique to analyze vortex formation time in several groups of patients with differing cardiac pathologies.

ABSTRACT:

Trans-mitral blood flow produces a three-dimensional rotational body of fluid, known as a vortex ring, that enhances the efficiency of left ventricular (LV) filling compared with a continuous linear jet. Vortex ring development is most often quantified with vortex formation time (VFT), a dimensionless parameter based on fluid ejection from a rigid tube. Our group is interested in factors that affect LV filling efficiency during cardiac surgery. In this report, we describe how to

use standard two-dimensional (2D) and Doppler transesophageal echocardiography (TEE) to noninvasively derive the variables needed to calculate VFT. We calculate atrial filling fraction (β) from velocity-time integrals of trans-mitral early LV filling and atrial systole blood flow velocity waveforms measured in the mid-esophageal four-chamber TEE view. Stroke volume (SV) is calculated as the product of the diameter of the LV outflow track measured in the mid-esophageal long axis TEE view and the velocity-time integral of blood flow through the outflow track determined in the deep transgastric view using pulse-wave Doppler. Finally, mitral valve diameter (D) is determined as the average of major and minor axis lengths measured in orthogonal mid-esophageal bicommissural and long axis imaging planes, respectively. VFT is then calculated as $4 \times (1-\beta) \times SV/(\pi D^3)$. We have used this technique to analyze VFT in several groups of patients with differing cardiac abnormalities. We discuss our application of this technique and its potential limitations and also review our results to date. Noninvasive measurement of VFT using TEE is straightforward in anesthetized patients undergoing cardiac surgery. The technique may allow cardiac anesthesiologists and surgeons to assess the impact of pathological conditions and surgical interventions on LV filling efficiency in real time.

INTRODUCTION:

Fluid mechanics is a critical yet often underappreciated determinant of left ventricular (LV) filling. A three-dimensional rotational body of fluid, known as a vortex ring, is generated whenever a fluid traverses an orifice[1-3]. This vortex ring improves the efficiency of fluid transport compared with a continuous linear jet[4]. Movement of blood through the mitral valve during early LV filling causes a vortex ring to form[5-8] and facilitates its propagation into the chamber by preserving fluid momentum and kinetic energy[9]. These actions enhance LV filling efficiency[4,10-13]. The ring not only inhibits blood flow stasis in the LV apex[14-17] but also directs flow preferentially beneath the anterior mitral leaflet[7,18], effects that decrease the risk of apical thrombus formation and facilitate filling of the LV outflow track[19], respectively. Contrast echocardiography[17], Doppler vector flow mapping[6,20,21], magnetic resonance imaging[7], and particle imaging velocimetry[9,22-24] have been used to demonstrate the appearance and behavior of trans-mitral vortex rings under normal and pathological conditions. The left atrial-LV pressure gradient, the degree of diastolic mitral annular excursion, the minimum LV pressure achieved during diastole, and the rate and extent of LV relaxation are the four major determinants of the duration, size, flow intensity, and position of the trans-mitral ring[2,12,25-29].

Vortex ring development is most often quantified with a dimensionless parameter (vortex formation time; VFT) based on fluid ejection from a rigid tube[3], where VFT is defined as the product of the time-averaged fluid velocity and the duration of ejection divided by the orifice diameter. The optimal size of a vortex ring is achieved when VFT is 4 *in vitro* because trailing jets and energetic limitations prevent it from attaining a larger size[3,4]. Mitral valve VFT has been approximated clinically using transthoracic echocardiography[8,30,31]. Based on analysis of trans-mitral blood flow velocity and mitral valve diameter (D), it can be easily shown[8] that $VFT = 4 \times (1-\beta) \times EF \times \alpha^3$, where β = atrial filling fraction, EF = LV ejection fraction, and $\alpha = EDV^{1/3}/D$, where EDV = end-diastolic volume. Ejection fraction is the ratio of stroke volume (SV) and EDV, allowing this equation to be simplified to $VFT = 4 \times (1-\beta) \times SV/(\pi D^3)$. Because VFT is dimensionless (volume/volume), this index allows direct comparison between patients of varying size without

adjustment for weight or body surface area[8]. Optimal VFT ranges between 3.3 and 5.5 in healthy subjects[8], and results are consistent with those obtained in fluid dynamics models[3,32]. VFT was shown to be ≤ 2.0 in patients with depressed LV systolic function, findings that are also supported by theoretical predictions[8]. Reductions in VFT independently predicted morbidity and mortality in patients with heart failure[30]. Elevated LV afterload[33], Alzheimer's disease[34], abnormal diastolic function[19], and replacement of the native mitral valve with a prosthesis[35] have also been shown to decrease VFT. Measurement of VFT may also be useful to identify blood flow stasis or thrombosis in patients with acute myocardial infarction[36,37].

Our group is interested in factors that affect LV filling efficiency during cardiac surgery[38-41]. We use standard two-dimensional and Doppler transesophageal echocardiography (TEE) to noninvasively derive the variables required to calculate VFT. In this report, we describe this methodology in detail and review our findings to date.

Protocol:

The Institutional Review Board of the Clement J. Zablocki Veterans Affairs Medical Center approved the protocols. Written informed consent was waived because invasive cardiac monitoring and TEE are routinely used in all patients undergoing cardiac surgery in our institution. Patients with relative or absolute contraindications for TEE, those undergoing repeat median sternotomy or emergency surgery, and those with atrial or ventricular tachyarrhythmias were excluded from participation.

1. Anesthesia

1.1. Provide each patient with intravenous midazolam (1 to 3 mg) and fentanyl (50 to 150 mcg) for conscious sedation before surgery.

1.2. Use local anesthesia (subcutaneous 1% lidocaine) for insertion of intravenous and radial artery catheters. Test the quality of the local anesthesia with a pinprick.

1.3. Ensure that the patient receives supplemental oxygen using a nasal cannula (2 to 4 L/min).

1.4. Place a central venous or pulmonary artery catheter using local anesthesia (subcutaneous 1% lidocaine) under sterile conditions through the right or left internal jugular vein with ultrasound guidance based on appropriate clinical indications.

1.5. Induce anesthesia using intravenous fentanyl (5 mcg/kg), propofol (1 to 2 mg/kg), and rocuronium (0.1 mg/kg). Maintain anesthesia using inhaled isoflurane (end-tidal concentration of 1%) in an air-oxygen mixture, fentanyl (1 to 2 mcg/kg/h), and rocuronium (0.05 mg/kg) titrated to effect using neuromuscular monitoring.

1.6. Suction the stomach using an oral-gastric tube.

1.7. Place ultrasound jelly in the patient's hypopharynx. Lift the jaw anteriorly and advance a TEE probe into the esophagus with gentle pressure to overcome resistance of the hypopharygeus muscle.

2. Transesophageal Echocardiography

2.1. Perform a comprehensive TEE examination following American Society of Echocardiography/Society of Cardiovascular Anesthesiologists guidelines[42] in each patient.

2.2. Place a pulse-wave Doppler sample volume between the tips of the mitral leaflets to record trans-mitral blood flow velocity in the mid-esophageal four-chamber TEE imaging plane (**Figure 1**).

2.3. Identify the early LV filling and atrial systole blood flow waveforms of trans-mitral blood flow velocity, and measure their corresponding peak velocities and velocity-time integrals (VTI_E and VTI_A, respectively) using the echocardiography equipment's integrated software package (**Figure 1**).

2.4. Calculate the atrial filling fraction (β) as the ratio of atrial to total LV filling:

$$\beta = \frac{VTI_A}{VTI_E + VTI_A}$$

2.5. Measure the maximum diameter of the LV outflow tract immediately below the aortic valve in the mid-esophageal aortic valve long axis TEE view during mid-systole (**Figure 2A**).

2.6. Calculate the area of the LV outflow tract assuming circular geometry as the product of $\pi/4$ and the square of the diameter (see step 2.5 above).

2.7. Obtain a deep transgastric long axis TEE view, and place a pulse-wave Doppler sample volume in the distal LV outflow tract to record a blood flow velocity envelope (**Figure 2B**) at the same level where the diameter was measured (see step 2.5 above); integrate the area of this waveform using the echocardiography equipment's software package to obtain VTI.

2.8. Multiply the resulting velocity-time integral (VTI) of the LV outflow track blood flow velocity waveform (**Figure 2B**) by the area of the outflow track (see step 2.6) to obtain stroke volume (SV).

2.9. Record video clips of the mid-esophageal bicommissural and LV long axis TEE imaging planes, respectively[42]. Be sure to include several cardiac cycles in each recording.

2.10. Visually inspect slow-motion images of the video clips (see step 2.9 above) after the ECG T-wave to choose the maximum opening of the mitral valve leaflets.

2.11. Measure the distance between the mitral leaflets (**Figures 3A** and **3B**) using the echocardiography equipment's "caliper" function.

2.12. Calculate the mitral valve diameter (D) as the average of the major (transcommissural anterior-lateral-posterior-medial) and minor (anterior-posterior) lengths.

2.13. Calculate VFT using the formula:

$$VFT = 4 \times (1 - \beta) \times \frac{\mathrm{SV}}{\pi D^3}$$

2.14. Perform all quantitative echocardiographic measurements in triplicate at end-expiration.

3. Experimental Design

3.1. Determine VFT, indices of LV diastolic function, and hemodynamics during steady-state conditions 30 minutes before and 15, 30, and 60 minutes after cardiopulmonary bypass (CPB) in 10 patients with normal preoperative LV ejection fraction under coronary artery surgery to test the hypothesis that CPB transiently decreases VFT[39].

3.2. Test the hypothesis that LV pressure-overload hypertrophy produced by aortic valve stenosis reduces VFT by examining (in one group of 8 patients undergoing aortic valve replacement) for severe aortic stenosis and comparing observations to another group of 8 patients with normal LV wall thickness undergoing coronary artery surgery[40]. Measure VFT, LV diastolic function, hemodynamics, and end-diastolic posterior wall thickness during steady-state conditions 30 minutes before CPB.

3.3. Test the hypothesis that abnormal diastolic blood flow entering the LV affects trans-mitral LV filling efficiency in 8 patients with aortic valve stenosis and moderate aortic insufficiency versus 8 patients with aortic stenosis who do not have regurgitant valves[38]. Measure VFT and other parameters as described above (step 3.2).

3.4. Test the hypothesis that advanced age is associated with a reduction in LV filling efficiency quantified using VFT in 7 octogenarians (82 ± 2 years) compared to 7 younger patients (55 ± 6 years)[41] undergoing coronary artery surgery. Ensure that both groups have normal preoperative LV ejection fraction. Measure VFT and other parameters as described above (step 3.2).

4. Statistics

4.1. Present the data as mean ± standard deviation.

4.2. Evaluate data using analysis of variance (ANOVA) followed by Bonferroni's modification of Student's *t*-test.

4.3. Use linear regression analysis to determine the relationships between VFT and end-diastolic posterior wall thickness and between VFT and age.

4.4. Reject the null hypothesis when $p < 0.05$.

Representative Results:

The current technique allowed us to reliably measure VFT during cardiac surgery under a variety of clinical conditions by obtaining each determinant from blood flow and dimensional recordings in standard TEE imaging planes. A pulse-wave Doppler sample volume was placed at the tips of the mitral leaflets in the mid-esophageal four-chamber view to obtain the trans-mitral blood flow velocity profile necessary to calculate atrial filling fraction (β; **Figure 1**). Stroke volume was determined using the continuity equation (velocity-time integral of the LV outflow track blood flow velocity waveform multiplied by the area of the outflow track) and LV outflow track diameter was measured in the mid-esophageal LV long-axis view (**Figure 2A**), whereas blood flow through the outflow tract was determined in the deep transgastric short axis imaging plane (**Figure 2B**). Finally, average mitral valve diameter was calculated as the average of major and minor axis diameters measured in the mid-esophageal bicommissural and LV long-axis planes (**Figures 3A** and **3B**, respectively). Measurement of VFT was associated with intra- and interobserver variability of 5% and 7%, respectively, similar to other indices of dimension and blood flow measured using TEE (data not shown). Using this technique, we first showed that exposure to CPB reduced VFT (5.3 ± 1.8 before *vs.* 4.0 ± 1.5 15 minutes after bypass, $p < 0.05$; **Figure 4**) in patients undergoing coronary artery surgery. VFT recovered to baseline values within 60 minutes after CPB. An increase in β (0.33 ± 0.04 before *vs.* 0.41 ± 0.07 15 minutes after CPB, $p < 0.05$) consistent with greater atrial contribution to LV filling was primarily responsible for the decline in VFT because SV and mitral valve diameter remained unchanged.

We also showed that a decrease in VFT occurs in patients with severe aortic valve stenosis and LV pressure-overload hypertrophy compared with those with normal LV wall thickness (3.0 ± 0.6 *vs.* 4.3 ± 0.5, respectively; $p < 0.05$; **Figure 5**). Early LV filling was attenuated (*e.g.*, E/A, 0.77 ± 0.11 compared with 1.23 ± 0.13; β, 0.43 ± 0.09 compared with 0.35 ± 0.02; $p < 0.05$ for each), and SV was reduced (72 ± 12 mL compared with 95 ± 10 mL; $p < 0.05$) in patients with *vs.* without LV hypertrophy; however, mitral valve diameter was similar between groups. A significant inverse correlation between VFT and posterior wall thickness (PWT) was shown with linear regression analysis ($VFT = -2.57 \times PWT + 6.81$; $r = 0.408$; $p = 0.017$). In addition, our results using this technique demonstrated that the presence compared to absence of moderate aortic insufficiency in patients with severe aortic valve stenosis increased VFT (5.7 ± 1.7 *vs.* 3.0 ± 0.6, respectively; $p < 0.05$; **Figure 5**) concomitant with a decrease in mitral valve diameter (2.2 ± 0.2 *vs.* 2.6 ± 0.1 cm, respectively; $p < 0.05$), whereas indices of LV diastolic dysfunction and SV were similar between groups. Finally, we were able to use our technique of measuring VFT to show that VFT was lower in octogenarians compared with younger patients (3.0 ± 0.9 *vs.* 4.5 ± 1.2; $p < 0.05$) concomitant with an impaired relaxation pattern of LV diastolic dysfunction (*e.g.*, E/A of 0.81 ± 0.16 *vs.* 1.29 ± 0.19; β of 0.44 ± 0.05 *vs.* 0.35 ± 0.03, $p < 0.05$ for each). A significant inverse correlation between VFT and age was also demonstrated ($VFT = -0.0627 \times age + 8.24$; $r = 0.639$; $p = 0.0139$; **Figure 6**).

FIGURE LEGENDS:

Figure 1: **Trans-mitral blood flow velocity waveforms.** Trans-mitral blood flow velocity waveforms during early LV filling (E) and atrial systole (A) obtained in the mid-esophageal four-chamber TEE view (left side of image); the area of each envelope was integrated using the equipment's software to obtain velocity-time integrals (right side of image) and the atrial filling fraction (β) was calculated. In this example, β = 4.28 cm/(4.28 cm + 6.73 cm) = 0.39 (see text).

Figure 2: Measurement of LV outflow track diameter. Measurement of LV outflow track diameter during mid-systole in the aortic valve long axis TEE view (**A**) (diameter = 2.23 cm); (**B**) blood flow velocity was measured in the in the distal LV outflow track using the deep transgastric long axis TEE view and the area of the resulting envelope (left side of panel B) integrated using the equipment's software to obtain a velocity-time integral (white arrow, right side of panel B). In this example, stroke volume = $\pi/4 \times (2.23\ cm)^2 \times 19.8\ cm = 77\ mL$ (see text).

Figure 3: Average mitral valve diameter was calculated as the average of major and minor axis diameters measured in the mid-esophageal bicommissural and LV long-axis planes. Mid-esophageal bicommissural (**A**) and LV outflow tract (**B**) TEE images were used to determine major (transcommissural anterior-lateral-posterior-medial) and minor (anterior-posterior) axis diameters, respectively. In this example, mitral valve diameter = (3.04 cm + 2.18 cm)/2 = 2.61 cm. This figure is reproduced with permission from Elsevier[38].

Figure 4: Temporal changes in VFT. Temporal changes in VFT before and 15, 30, and 60 minutes after cardiopulmonary bypass (CPB) in patients undergoing coronary artery surgery; *indicates significant ($p < 0.05$) difference from the "before CPB" measurement.

Figure 5: Effects of LV pressure-overload hypertrophy resulting from severe aortic valve stenosis in the absence (-) or presence (+) of moderate aortic insufficiency (AI) in patients undergoing aortic valve replacement. Patients with normal LV wall thickness undergoing coronary artery surgery served as controls (normal). *Significantly ($p < 0.05$) different from normal; †Significantly ($p < 0.05$) different from both normal and hypertrophy-AI.

Figure 6: Correlation between age and VFT in 14 patients undergoing coronary artery surgery. VFT = -0.0627 × age + 8.24; r = 0.639; p = 0.0139.

DISCUSSION:

The current results illustrate that VFT can be reliably measured during cardiac surgery using the TEE techniques described here. Previous descriptions of VFT used transthoracic echocardiography in conscious subjects, but this approach cannot be utilized when the chest is open. We used intraoperative TEE to determine VFT in the anesthetized patients undergoing cardiac surgery during which changes in LV filling dynamics are often encountered as a result of ischemia-reperfusion injury or surgical interventions. Our findings indicate that VFT measurements reflect changes in LV filling efficiency produced by transient CPB-induced impaired relaxation pattern diastolic dysfunction, aortic valve disease, and aging. The current

technique for calculating VFT during cardiac surgery requires high-quality TEE images and video clips during steady-state hemodynamic conditions to assure precise measurements of mitral valve and LV outflow tract dimension and blood flow (**Figures 1, 2,** and **3**). Not all patients will have optimal imaging windows because of off-axis rotation of the heart or pathological changes in cardiac geometry. Despite these potential limitations, experienced intraoperative echocardiographers should be able to easily obtain the necessary mid-esophageal four-chamber, mid-esophageal bicommissural, mid-esophageal LV long axis, and deep transgastric long axis views during the comprehensive TEE examination[42]. The technique may also be unreliable when rapidly changing hemodynamic conditions are present. It does not provide direct visualization of blood flow movement within the LV associated with the vortex, as previously characterized using Doppler vector flow mapping[6,20,21] or particle imaging velocimetry[9,22-24]. Accurate measurement of LV outflow track diameter using two-dimensional echocardiography is especially important because this variable is squared in the calculation of area and errors are magnified as a result. Similarly, accurate measurements of mitral valve minor and major axis length are essential because the cube of the average of these two dimensions appears in the denominator of the VFT formula. Two-dimensional echocardiography consistently underestimates aortic and mitral valve areas compared with three-dimensional reconstruction techniques[43, 44]. The impact of these differences between two- and three-dimensional TEE on VFT is an area of current research by our group.

Additionally, isoflurane was used for maintenance of anesthesia in our studies. This volatile anesthetic is a vasodilating negative inotrope that reduces LV preload and afterload, decreases myocardial contractility, and affects LV diastolic function in a dose-related manner[45,46]. These cardiovascular changes may have influenced atrial filling fraction and stroke volume in our studies. Nevertheless, the values of VFT obtained in anesthetized patients with normal preoperative LV ejection fraction undergoing coronary artery surgery before CPB were similar to those described in healthy conscious subjects[8]. These data suggest that baseline anesthesia does not substantially alter LV filling efficiency, but we are currently examining this hypothesis. VFT has been previously shown to be an independent predictor of mortality in patients with congestive heart failure[30], but it is unknown whether intraoperative changes in VFT are predictive of perioperative morbidity or mortality in cardiac surgery patients. This topic is also an area of interest that we are actively pursuing.

We first used this technique of noninvasively calculating VFT in a study examining the impact of CPB on VFT in isoflurane-fentanyl-anesthetized patients with normal preoperative LV ejection fraction undergoing coronary artery surgery[39]. LV diastolic dysfunction occurs after cardiopulmonary bypass as a result of global ischemia-reperfusion injury and a profound systemic inflammatory response[47-49]. This diastolic dysfunction eventually recovers within minutes to hours based on the efficacy of myocardial protection during and the duration of CPB[50]. Indeed, our findings confirmed that LV diastolic dysfunction occurs after CPB. This effect was accompanied by transient reductions in VFT that recovered within one hour after separation from CPB. The declines in VFT resulted from an increase in β and a modest decrease in SV because mitral valve diameter was unchanged. The recoveries of VFT, β, E/A, and SV after CPB were similar. Notably, VFT observed here did not fall below the normal range of VFT (3.3 to 5.5) in

healthy individuals. Our patients had normal preoperative LV systolic and diastolic function, were exposed to relatively short CPB times (93 ± 27 min), and were treated with regular doses of antegrade and retrograde cardioplegia. These factors probably combined to reduce ischemia-reperfusion injury during aortic cross-clamp application[39]. CPB has also been shown to cause transient declines in trans-mitral blood flow propagation velocity (V_p) consistent with attenuated early LV filling in patients undergoing coronary artery surgery[49] as a result of decreases in LV compliance[51] and reductions in early diastolic intraventricular pressure gradients[52]. A relationship between vortex ring formation and V_p was previously demonstrated[53], and our findings supported those of other investigators[49] in similar patient populations.

We subsequently examined the effects of pressure-overload LV hypertrophy produced by severe calcific degenerative aortic valve stenosis in patients with preserved LV systolic function undergoing aortic valve replacement[40]. A second group of patients with normal LV wall thickness undergoing coronary artery surgery served as controls. Chronically elevated LV end-systolic wall stress causes LV pressure-overload hypertrophy as a compensatory response in the presence of aortic valve stenosis[54]. LV wall thickening without dilatation occurs as a consequence of an increase in the diameter of individual myocytes. This LV remodeling is associated with interstitial fibrosis[55,56]. Delays in apical recoil[57,58] also occur that further attenuate early LV filling[58,59], which causes LV diastolic dysfunction by delaying LV relaxation and reducing LV compliance[55,60]. Thus, VFT is reduced in the presence of delayed relaxation in patients with LV pressure-overload hypertrophy *vs.* those with normal LV wall thickness. Our findings were attributed to an increase in β and decline in SV at similar filling pressures consistent with a decrease in LV compliance. A significant correlation between decreases in VFT and the severity of hypertrophy was shown using linear regression analysis. This observation suggests that the degree of pressure-overload hypertrophy is inversely related to LV filling efficiency quantified using vortex formation time.

Valvular insufficiency often occurs in conjunction with severe calcific degenerative aortic valve stenosis because prominent leaflet calcification prevents complete coaptation. We conducted another investigation to ascertain whether regurgitant blood flow into the LV through an incompetent aortic valve affects LV filling efficiency by interfering with trans-mitral blood flow[38]. We compared patients with severe aortic valve stenosis undergoing valve replacement who had moderate centrally-directed aortic insufficiency with a second group of patients who did not have regurgitation. We quantified aortic insufficiency using the regurgitant jet width LV outflow track diameter ratio measure with color Doppler M-mode echocardiography[61]. Our results showed that moderate aortic insufficiency increases VFT in patients with aortic valve stenosis. However, this increase in VFT does not suggest an improvement in LV filling efficiency has occurred because of abnormal regurgitant flow into the LV through the aortic valve. LV diastolic pressure rapidly increases in moderate to severe aortic insufficiency[62], attenuating trans-mitral LV filling and reducing mitral valve area[63-65]. The results indicate that mitral valve diameter and area were reduced in patients with moderate aortic insufficiency *versus* those without regurgitation. These observations were most likely due to a decrease in minor axis length, resulting from attenuated anterior mitral leaflet opening caused by aortic regurgitant during LV filling, thereby, falsely elevated VFT. Indeed, VFT reported in our study (5.7 ± 1.7) was greater than the upper limit of normal VFT (5.5) in healthy conscious individuals[8] and patients with normal LV geometry during

anesthesia (4.3 ± 0.5)[40]. Therefore, it is highly likely that abnormal diastolic flow into the LV invalidates VFT as an index of LV filling efficiency.

We recently studied the influence of advanced age on VTF in elderly patients undergoing coronary artery surgery[41]. Progressive LV diastolic stiffening[66], decreased intraventricular diastolic kinetic energy[67], and attenuated diastolic suction[68] cause LV diastolic function in the elderly[69-72]. Octogenarians with normal preoperative LV ejection were compared with a younger cohort of patients (≤ 62 years of age). We found that VFT was lower in octogenarians compared with younger patients. These observations were expected and occurred in conjunction with an impaired relaxation pattern of LV diastolic dysfunction and a modest reduction in SV at similar LV filling pressures. Mitral valve diameter was similar in octogenarians *versus* younger patients and did not contribute to the differences in VFT between groups. It is noteworthy that VFT was similar in octogenarians compared with patients with severe aortic valve stenosis that we previously reported[38,40]. Indeed, aortic stenosis is another condition characterized by impaired relaxation LV diastolic dysfunction and reductions in LV compliance. A significant inverse correlation between VFT and age was also demonstrated despite the small sample size (n = 7 per group; **Figure 6**). The decline in VFT that occurs with age that may eventually become indistinguishable from heart failure produced by pathological processes such as restrictive diastolic dysfunction[19] or dilated cardiomyopathy[8]. Our results were consistent with reductions in early peak diastolic intraventricular kinetic energy in elderly subjects with depressed LV function[67].

In summary, noninvasive measurement of VFT using standard two-dimensional and Doppler TEE is straightforward in anesthetized patients undergoing cardiac surgery. This technique may allow cardiac anesthesiologists and surgeons to assess the impact of pathological conditions and surgical interventions on LV filling efficiency in real time.

Acknowledgements:
This material is the result of work supported with resources and the use of the facilities at the Clement J. Zablocki Veterans Affairs Medical Center in Milwaukee, Wisconsin.

Disclosures:
The authors have no competing financial interests or other conflicts of interest pursuant to this work.

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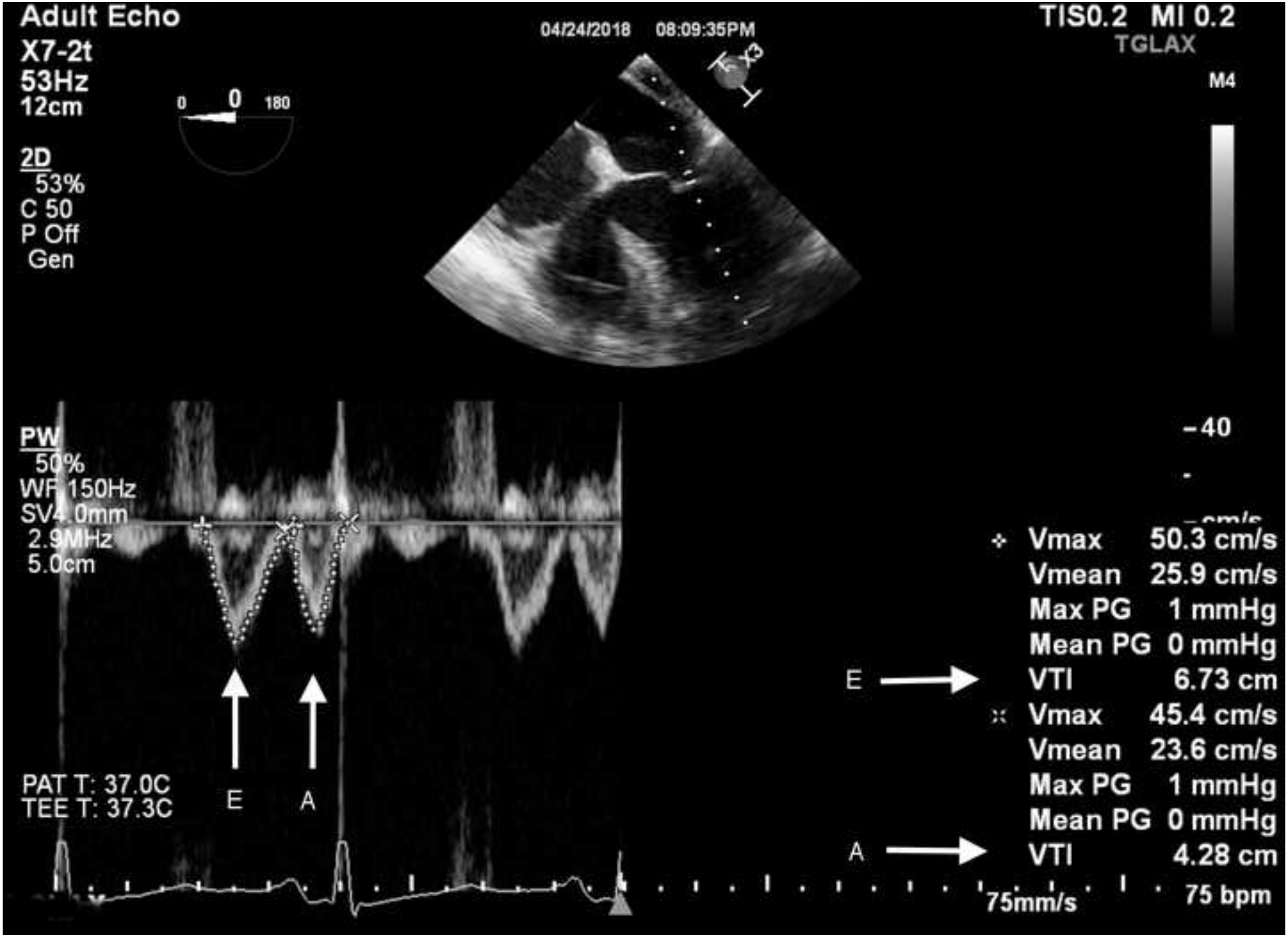

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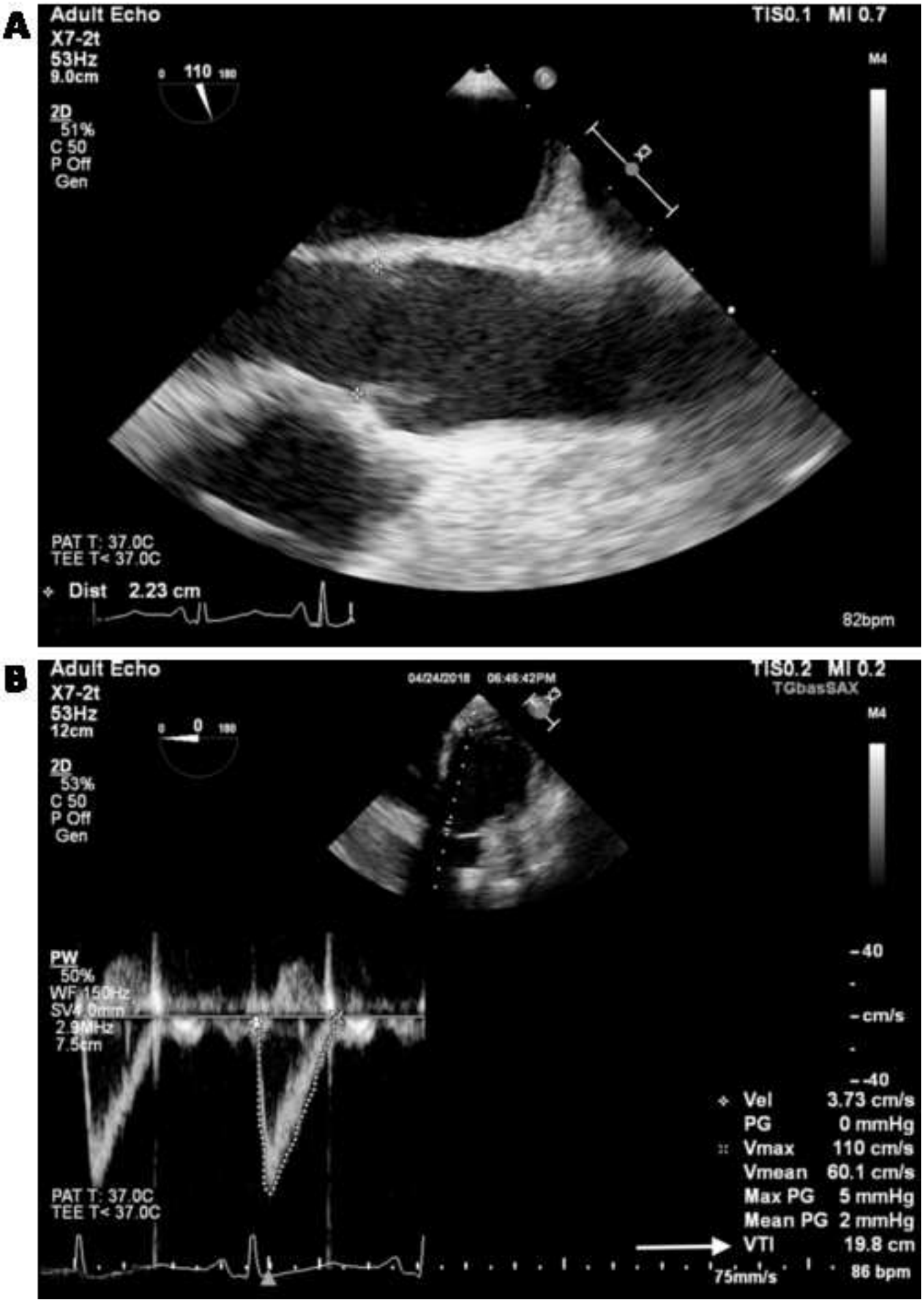

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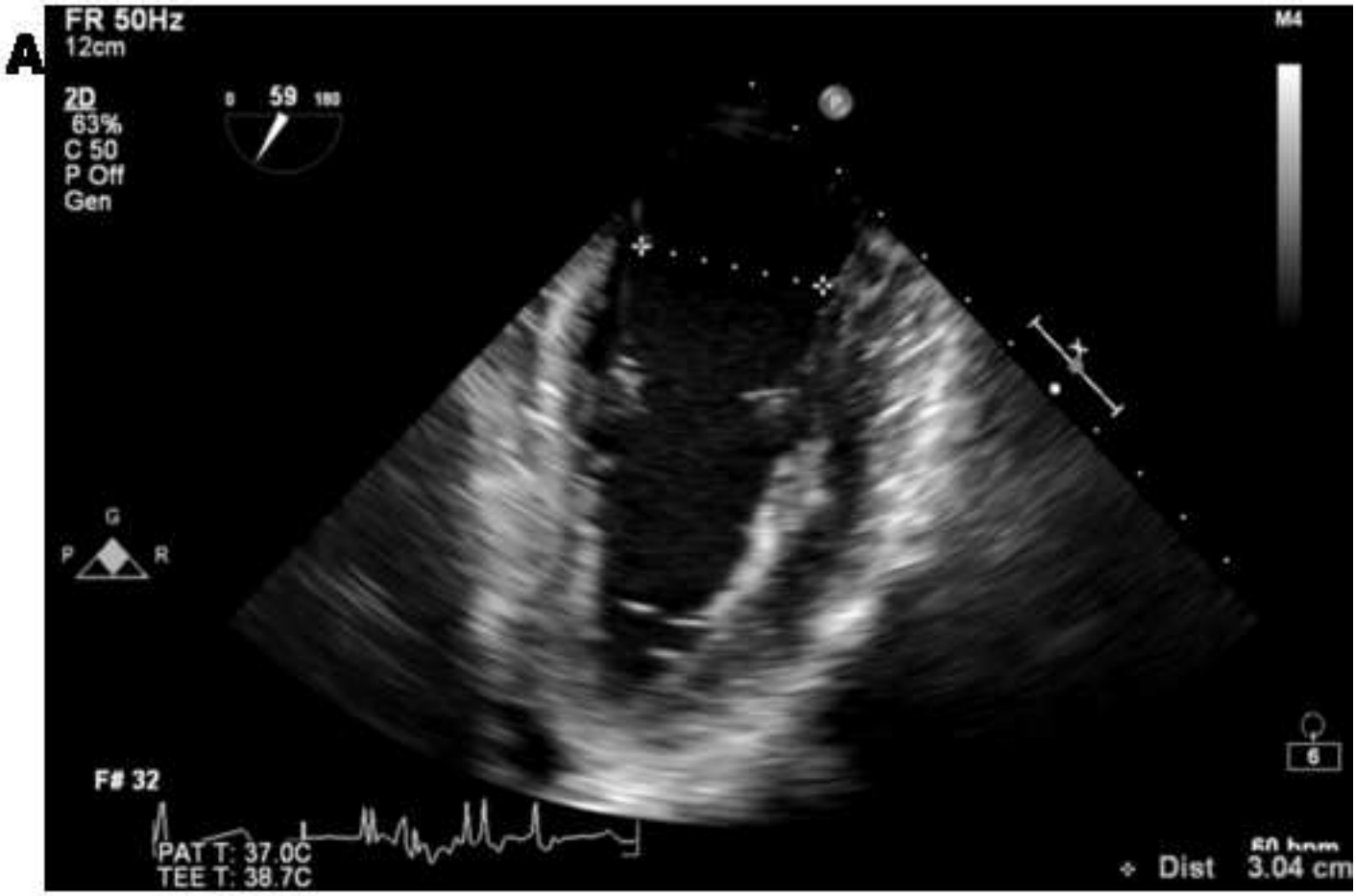

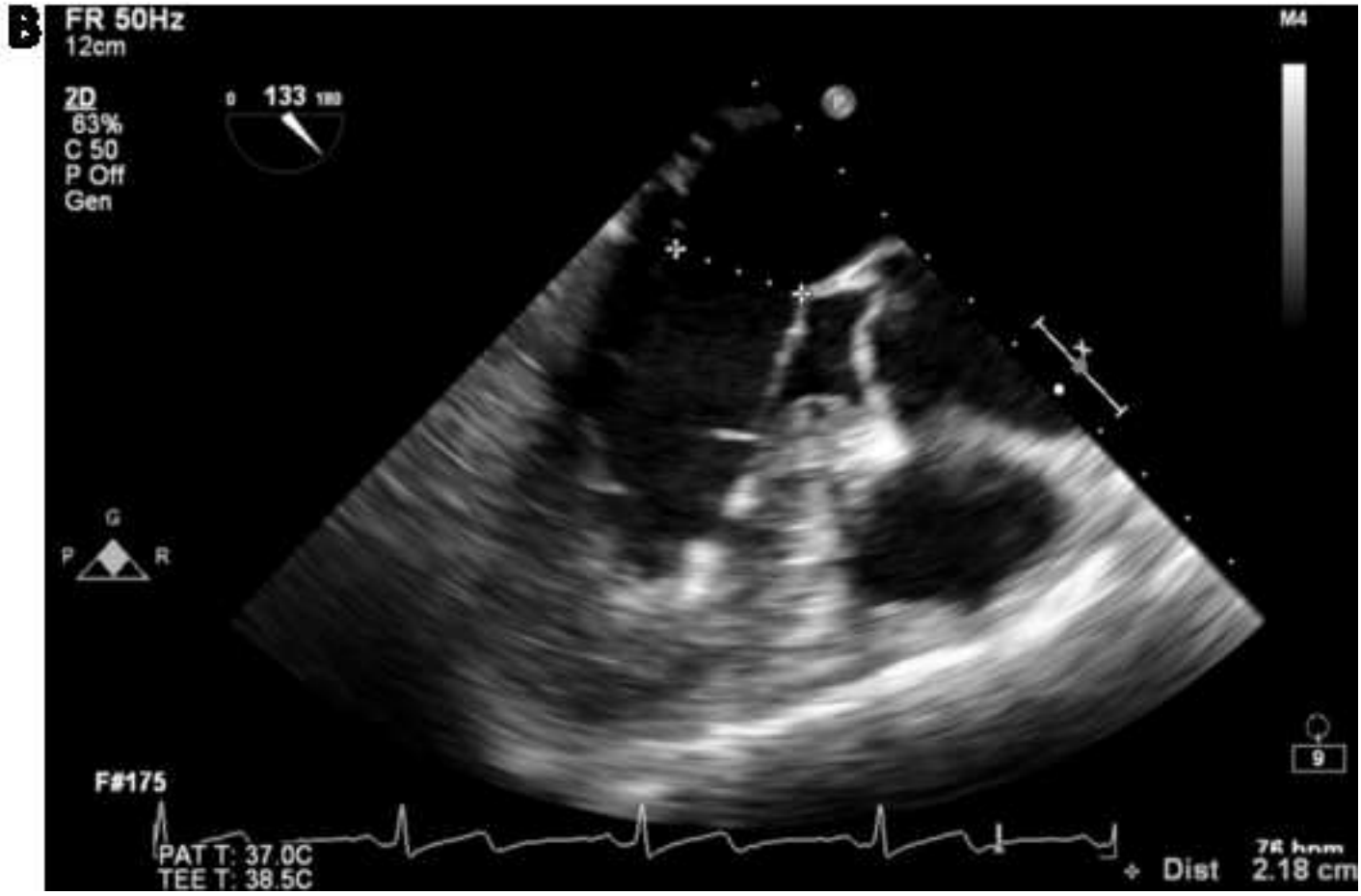

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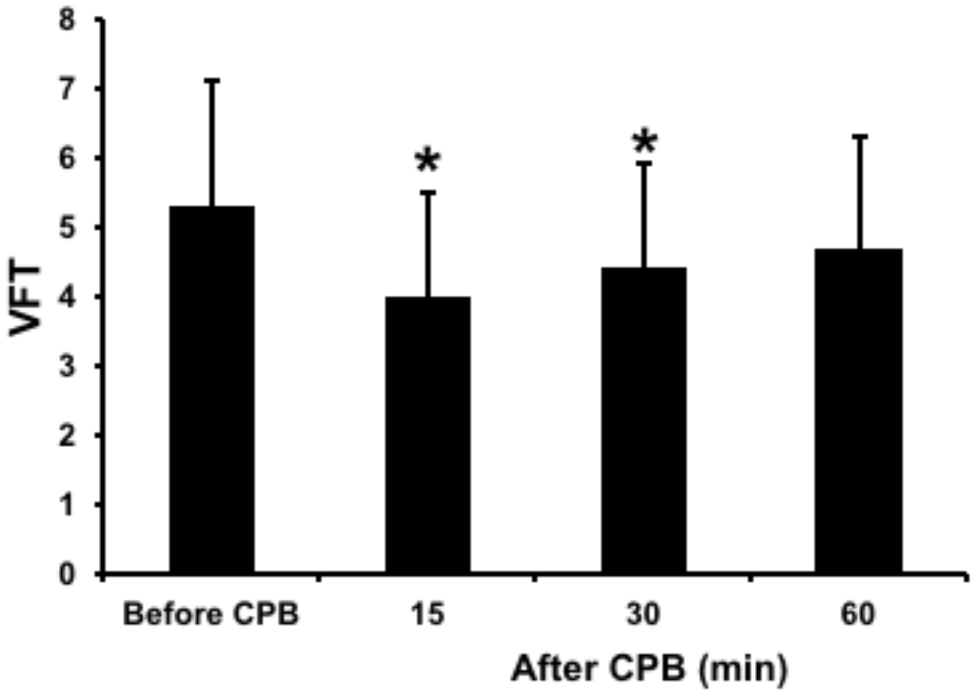

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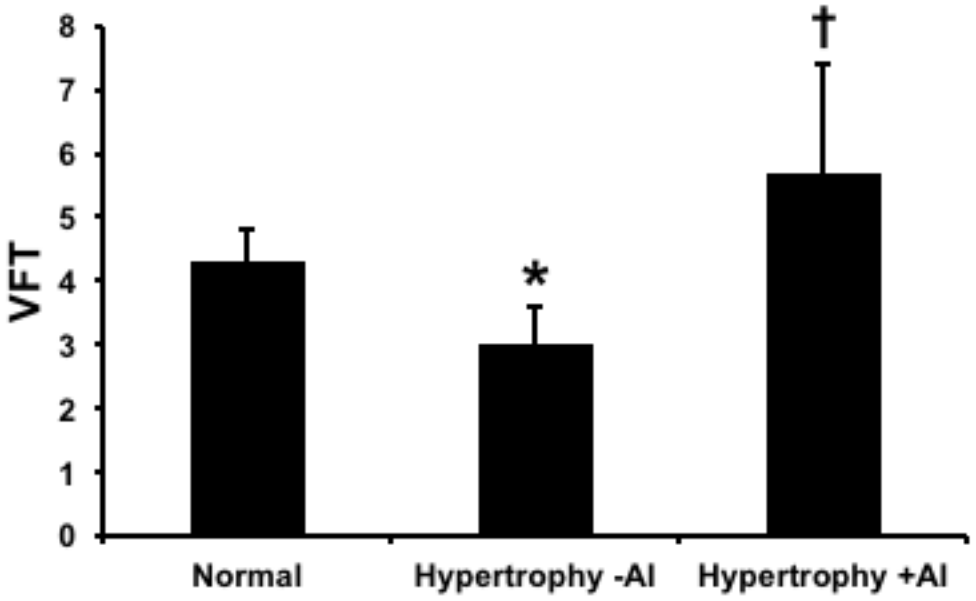

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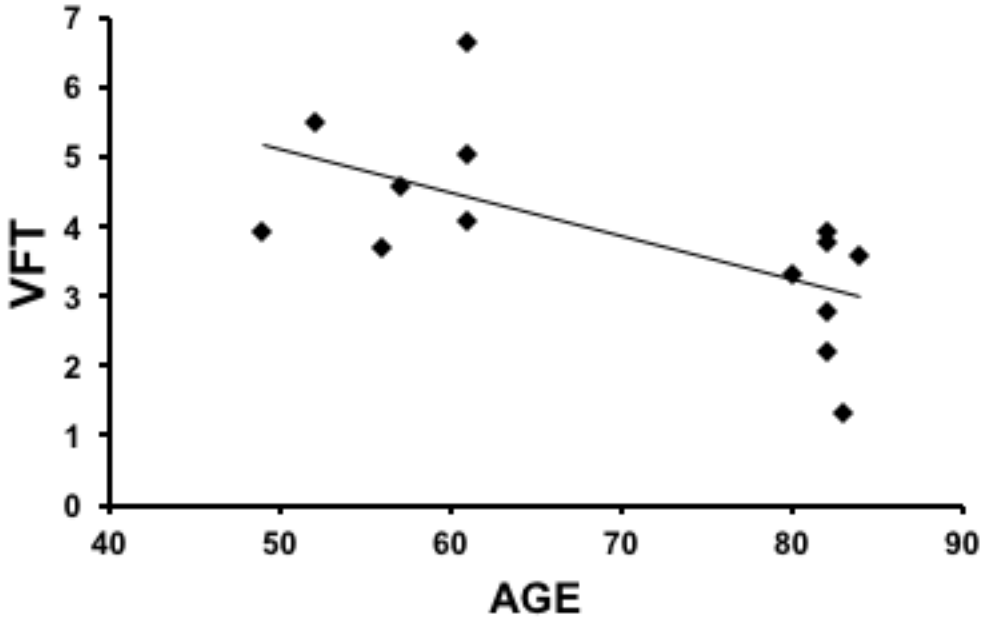

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Article Title: NON-INVASIVE EVALUATION OF VORTEX FORMATION TIME ...

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Responses to Editor and Reviewer Comments

Editor

1. I've proofread the manuscript to be sure that it is entirely correct. There are no spelling or grammatical errors.
2. These sections have been revised.
3. These changes have been made.
4. I have no idea how to accomplish this task nor do I know anyone who knows how to do it. I've left the figures in separate panels as a result.
5. The title has been simplified as requested.
6. An email address for each author has been included in the revised manuscript.
7. I've added the following short abstract as requested: "Here, we present a protocol to measure vortex formation time, an index of left ventricular filling efficiency, using standard transesophageal echocardiography techniques in patients undergoing cardiac surgery. We apply this technique to analyze vortex formation time in several groups of patients with differing cardiac pathologies."
8. The Abstract has been rewritten to emphasize the methodology as requested.
9. The numbering of the Protocol has been adjusted to follow the JoVE Instructions for Authors as requested.
10. Spacing has been adjusted throughout the manuscript as requested.
11. All commercial language has been removed from the manuscript as requested. The Table of Materials and Reagents has been updated.
12. The protocol has been rewritten in imperative tense as requested.
13. Additional information has been added to the protocol as requested.
14. The dosages of all medications are now specified in the revised text.
15. Local anesthesia was administered subcutaneously using 1% lidocaine and its efficacy verified using a pinprick. This information has been added to the text as requested.
16. Supplemental oxygen was provided using a nasal cannula. This information has been added to the text as requested.
17. Additional details were added as requested.
18. The text was revised to include a description of how parameters were calculated.
19. The Representative Results section has been reviewed as requested. I've commented about how reproducible results were obtained using measurements of cardiac dimension and blood flow in several standard, easily obtained, transesophageal imaging planes. I've discussed figures 1, 2, and 3 in the text in this context.
20. I revised the Discussion to include commentary about the protocol, modifications/troubleshooting the technique, limitations to the technique, significance, and potential future applications as requested.
21. An acknowledgements section has been added as requested.
22. A disclosures section has been added as requested. The authors have competing financial interests or other conflicts of interest pursuant to this work.
23. The references have been reformatted as instructed.
24. See response above. The references have been changed to comply with the journal's style.
25. The table of essential supplies, reagents, and equipment has been revised as requested.

Reviewer #1

Major Comments

1. The reviewer is correct that we did not visualize blood flow movement in the LV. This limitation has been inserted in the revised manuscript. The reviewer is also correct that transthoracic echocardiography cannot be performed during cardiac surgery in the presence of an open chest (this requires an epicardial approach instead); I've also commented on this limitation in the revised manuscript.

2. I respectively disagree with the reviewer's assertion. The rapid increase in LV pressure that occurs in aortic insufficiency is known to attenuate early LV filling (see references #63-65). Our data indicate that mitral valve minor axis diameter was reduced in this setting, whereas atrial filling fraction and stroke volume (the other determinants of VFT) were unaffected (see reference #40).

Minor Comments

1. I'm not sure what to make of this comment. There doesn't appear to be a question here. The control values of VFT in patients with coronary artery disease and normal LV ejection fraction was similar those previously reported in conscious healthy subjects (see reference #8).
2. The sample sizes are small, but power analyses indicated that the sample sizes were appropriate for the purposes of meaningful statistical comparison (see "statistical analysis" sections of references #38-41).
3. We did not conduct tissue Doppler analysis and cannot calculate E/e' as an estimate of left atrial filling pressure as a result. Diastolic function was graded in the original papers (see references #38-41for details) using transmitral and pulmonary venous blood flow velocity profiles. This is standard practice in the operating room.
4. Measurement of diastolic dysfunction was not the primary objective of the current work or that of the cited papers. Transmitral blood flow velocity patterns were obtained to calculated atrial filling fraction. As mentioned, we also reported pulmonary venous blood flow velocity profiles in the original papers.
5. The reviewer should refer to the primary manuscripts (references #38 and 40) for details. The mitral valve dimension measurements to which the author refers are located in table 2 of each of these papers.
6. I respectfully disagree with this contention. The power analysis indicated that the sample size was adequate for statistical analysis. The regression analysis also provided a statistically inverse significant correlation between posterior wall thickness and VFT in patients with aortic valve stenosis (see reference #40).
7. The concluding sentence has been qualified with this potential limitation in mind.

Reviewer #2

Thank you for your kind comments about our manuscript. I've revised the Introduction modestly to include the reviewer's two suggested citations.

Reviewer #3

Major Concerns

1. Kheradvar *et al* (reference #19) examined the relationship between VFT and E/A. They demonstrated that VFT is decreased in delayed relaxation, pseudonormal, and restrictive diastolic dysfunction. This topic was discussed in our other reports (references #38-41), and I'd prefer not to repeat it here (the readers can refer to these papers for additional details as desired). To my knowledge, VFT has not been directly compared with e' or other tissue Doppler indices.

2. It is clear that VFT is an independent predictor of outcome in patients with heart failure (see reference #30). Whether VFT predicts morbidity or mortality in patients undergoing cardiac surgery is not clear at present, but we are conducting studies to address this hypothesis, as mentioned in the revised Discussion.

Minor Concerns

None.

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