New insight into the intraventricular pressure gradient as a sensitive indicator of diastolic cardiac dysfunction in patients with childhood cancer after anthracycline therapy

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Abstract

Cardiac dysfunction due to cardiotoxicity from anthracycline chemotherapy is a leading cause of morbidity and mortality in survivors of childhood cancer. The intraventricular pressure gradient (IVPG) of the left ventricle (LV) is the suction force of blood from the left atrium to the LV apex during early diastole and is a sensitive indicator of diastolic function. We assessed IVPG as a new indicator of the cardiac dysfunction in survivors of childhood cancer after anthracycline therapy. We performed a prospective echocardiographic study on 40 survivors of childhood cancer aged 6-26 years who received anthracycline therapy (group A) and 53 similar-age normal controls (group N). The subjects were divided into the younger groups, N1 and A1 (age <16 years); and older groups, N2 and A2 (age ≧16 years). IVPG was calculated using color M-mode Doppler imaging of the mitral inflow using Euler's equation. Total IVPG was divided into the basal and mid-to-apical IVPG to demonstrate more clearly the mechanisms of the LV diastolic suction force. The total anthracycline dose was 16.2-600.0 mg/m^2 (median 143.5 mg/m^2). Total IVPG significantly decreased in group A2 compared with that in group N2 (0.39 $\pm$ 0.07 vs. 0.29 $\pm$ 0.11 mmHg/cm; p = 0.010). The mid-to-apical IVPG significantly decreased in groups A1 and A2 compared with that in groups N1 and N2, respectively (N1 vs. A1: 0.20 $\pm$ 0.05 vs. 0.16 $\pm$ 0.05 mmHg/cm, p = 0.036; N2

vs. A2: 0.21 ± 0.06 vs. 0.14 ± 0.06 mmHg/cm, p = 0.001). Basal IVPG, E wave, and E/e' were not significantly different between patients and normal controls. The total and mid-to-apical IVPG, especially mid-to-apical IVPG, could be sensitive new indicators in survivors of childhood cancer after anthracycline therapy.

Key words: intraventricular pressure gradient, childhood cancer, anthracycline, cardiotoxicity, diastolic function

Introduction

The mortality rate among patients with cancer has decreased over the past three decades. In patients with childhood cancers, cardiotoxicity by cancer therapy has become a leading cause of morbidity and mortality [1, 2, 3]. Anthracycline is the main cause of cardiotoxicity. When heart failure occurs, the mortality rate reaches even 60% in 2 years [4]. The conventional definition of cardiac dysfunction by cancer therapy was based on the left ventricular ejection fraction (LVEF) [5]. However, when the EF decreases during or within 1 year of chemotherapy, it is often too late for recovery, and subsequent heart failure and early death can occur unless early medication is administered. [6]. Recently, cardiac dysfunction by cancer therapy has been detected earlier by using speckle tracking imaging [5, 7, 8]. In fact, the global longitudinal strain (GLS) was improved by administering β-blockers in patients with a decreased GLS, even if they had a normal EF after cancer therapy [9]. Therefore, early detection and treatment of cardiotoxicity is important.

Although diastolic dysfunction has been suggested to precede systolic dysfunction in patients with various conditions [10], 32.1% of survivors with a normal three-dimensional LVEF had evidence of cardiac dysfunction, using a GLS (28.0%); and only 8.7% of patients were determined to have diastolic dysfunction according to the

recommendations for the evaluation of LV diastolic function by American Society of Echocardiography [11] in a long-term follow up study with a median follow-up period of 31 years [12]. These data indicate that the conventional methods are inadequate, and new methods are needed to diagnose diastolic dysfunction at the early stage in childhood cancer survivors. Although, there has been a lack of evidence on the ability of echocardiographic parameters to predict late cardiotoxicity during long-term follow-up. Measuring the intraventricular pressure difference (IVPD) during early diastole is a new method to detect diastolic function. IVPD is the suction force, which sucks blood from the left atrium (LA) into the left ventricle (LV) during early diastole. It plays a fundamental role in diastolic function [13]. IVPD is also a noninvasive measurement method when echocardiography used. Furthermore, it correlates with the tau index, which is the gold standard for determining diastolic function and can only be measured with invasive techniques [14]. When cardiac function is assessed in the pediatric population, the size of the heart represents an important issue to be considered, as the LV in adolescents and adults is almost twice as long as the LV in infants. Popović et al. described IVPG as IVPD divided by LV length [15] and showed that, although IVPG is not completely independent from the size of the heart, it is less dependent on it than IVPD [15]. Furthermore, we recently published an article, [16] which demonstrated that

total, basal, and mid-to-apical IVPDs were significantly correlated with age and the parameters of heart size in healthy infants to adolescents, corresponding to the previous paper. [15] Interestingly, mid-to-apical IVPG correlated with age and e' positively, even after adjustment for left ventricular length. These findings indicated that the suction force increased at the mid-to-apical segment, correlating with increasing heart size and developing left ventricular relaxation as well as the possibility of its usefulness to assess diastolic function. Despite the usefulness of the IVPG, no report has assessed diastolic function using IVPG in survivors of childhood cancer. Therefore, the aim of this study is to assess the usefulness of the IVPG as a sensitive indicator for detecting diastolic cardiac dysfunction in survivors of childhood cancer after anthracycline therapy.

Materials and Methods

Study subjects

We performed a prospective echocardiographic study on 40 survivors of childhood cancer aged 6-26 years who received anthracycline therapy at the Department of Pediatrics of Juntendo University Hospital between June 1988 and March 2016 (group A) and 53 similar-age, normal controls (group N). Echocardiographic study was performed at least 1 year after the completion of anthracycline therapy. In group A, the

exclusion criteria were: (1) congenital heart disease, (2) valve regurgitation or stenotic disease, and (3) chromosomal disease (for example 21 trisomy).

Age-matched healthy individuals were recruited from Juntendo University and Shizuoka Children's Hospital as normal controls for IVPG analysis; these were either healthy volunteers or children undergoing echocardiography for the evaluation of innocent murmurs. They had no history of cardiovascular disease and showed normal sinus rhythm on ECG and normal findings on echocardiography. All participants or their guardians provided written informed consent, as established by Institutional Review Board of Juntendo University and Shizuoka Children's Hospital. In children, cardiac function dramatically changes with age [15]. Therefore, to identify the characteristics of myocardial deformation in childhood cancer more clearly, all subjects were divided into the younger groups, N1 and A1 (age <16 years); and older groups, N2 and A2 (age ≧16 years).

Echocardiography

Echocardiography was performed using a GE Vivid E9 ultrasound system (GE Healthcare, Milwaukee, WI, USA) with an S6 or M5 probe as appropriate for patient size. For each plane, three consecutive cardiac cycles were acquired during a breath

hold at end expiration, if possible. In younger children, we selected three cardiac cycles at end expiration on the respiratory trace. Echocardiography was performed by 2 experienced observers (S.S. and K.T.).

Measurement of the conventional parameters of echocardiography

The LV end diastolic volume, LV end-systolic volume, and LVEF were calculated with the modified Simpson's method using apical two- and four-chamber views. Three LV short-axis planes at the basal, mid, and apical levels, as well as an apical four-chamber view, were acquired at rates of 75–115 frames/sec and stored digitally for offline analysis using the EchoPAC system version 108.1.4 (GE Healthcare, Milwaukee, WI, USA). The mitral inflow E-wave, A-wave, and E/A ratio were measured using pulse-wave Doppler. The peak myocardial velocities during early diastole (e') were measured at the mitral lateral and septal annulus using tissue Doppler imaging. The isovolumic relaxation time (IVRT) was also measured as per the usual manner.

Measurements of the parameters of myocardial deformation

The circumferential strain (CS) and longitudinal strain (LS) were analyzed by using EchoPAC, as described previously [17]. CS was analyzed by segmental legion; basal,

papillary, and apical from the slice of short-axis view [18] -to make more clearly the mechanisms and correlation of the LV myocardial deformation and diastolic suction force.

Measurement of the IVPD and IVPG

Color M-mode images were obtained by apical four-chamber view that was used to assess LVEF and LV longitudinal deformation [19], and analyzed with an in-house code that was written in MATLAB (The MathWorks, Natick, MA, USA) using the following image-processing algorithm (Fig. 1):

$$(\partial P)/(\partial s) = -\rho\ ((\partial v)/(\partial t) + v\ (\partial v)/(\partial s)) \quad (1)$$

The images were reconstructed using a de-aliasing technique. In Equation (1), *P* is the pressure, ρ is the constant blood density (1060 kg/m^3), *v* is the velocity, *s* is the position along the streamline of the transmitral flow that was measured with the color Doppler M-mode line, and *t* is the time. The relative pressures within the region of interest can be calculated from the reconstructed velocity field [19]. The pressure difference at each point along the streamline from mitral valve to the LV apex was measured as described

previously [20]. This method has been validated through a comparison of these values with direct measurements using micromanometers [19]. All data were measured using at least three beats, and the mean values were used for the final analysis. Because the length of the LV is almost twice as large in adults as in children, we calculated the IVPG as:

IVPG = IVPD divided by the LV length [15]

The total IVPG was divided into the basal and mid-to-apical IVPG (Fig. 1). The basal segment was defined as the first one-third of the total LV length from the mitral valve, and the mid-to-apical segment was defined as the remaining two-thirds as previously described [20].

Statistical analysis

Data with normal distributions are expressed as means ± standard deviations. Data with non-normal distributions are expressed as median (25th and 75th percentiles).

After evaluating the data for normality, all group differences were assessed using one-factor analysis of variance with a post hoc Tukey–Kramer comparison test for data with

normal distributions or the Steel–Dwass test for data with non-normal distributions in each age group. Pearson's chi-square test was used for categorical variables. Student's t-test was examined for gender difference in each group.

The correlations between IVPGs and each variable were evaluated using Pearson's correlation coefficient (r), after non-normal distributed data were transformed to normal distribution by logarithmic or Johnson normalization.

To assess the inter- and intraobserver agreement for the total, basal, and mid-to-apical IVPG, we examined 14 randomly selected subjects in the same manner. Two independent observers analyzed the same images, and one blinded observer repeated the analysis on a separate day more than 1 month after the first measurements were taken. The inter- and intraobserver agreements were evaluated using the coefficient of variation (i.e., the standard deviation of the difference of paired samples that were divided by the average of the paired samples), intraclass correlation, and mean absolute relative difference. Statistical significance was set at $p < 0.05$. The analyses were performed using JMP® version 13.0 (SAS Institute Inc., Cary, NC, USA).

Results

Patients

Table 1 summarizes the demographic data of the patients and normal controls. We included 40 survivors of childhood cancer aged 6-26 years who received anthracycline treatment (group A) and similar-age normal controls (group N). In group A, the diagnoses included leukemia (n=19), lymphoma (n=7), Wilms tumor (n=5), neuroblastoma (n=2), hepatoblastoma (n=2), rhabdomyosarcoma (n=2), Ewing's sarcoma (n=2), and granulocytic sarcoma (n=1). These patients underwent chemotherapy included anthracycline therapy between June 1988 and March 2016. Echocardiography was performed at a mean of 8.0 years (range, 1.1-15.8 years) after the completion of anthracycline therapy. The median cumulative anthracycline dose was 143.5 mg/m^2 (60.0, 214.7 mg/m^2) and only five patients received a dose greater than 300 mg/m^2. Four patients in group A1 and 5 patients in group A2 received radiotherapy that involved the heart. The mean cumulative radiation dose that involved the heart was 26.0±13.9 (range: 12 to 48 Gy) in group A1 and 24.75±14.36 (range: 12 to 45 Gy) in group A2. Two patients underwent allogeneic stem cell transplantation. There was no difference in IVPG between these 2 patients and the other patients. The New York Heart Association grade was 1 in all subjects in group A. None had overt clinical heart failure at the time of the study.

Basic echocardiographic measurements

Table 2 summarizes the echocardiographic parameters in patients and normal controls. LVEF, E and A wave velocity (m/sec), E/A velocity ratio, e', E/e', IVRT, apical CS, and LS, were not significantly different between patients and normal controls. Basal CS was significantly worse in subjects in groups A1 and A2 than in those in groups N1 and N2. Papillary CS was significantly worse in group A2 than in those in group N2.

IVPG measurements

Total IVPG was significantly worse in subjects in group A2 than in those in group N2. Mid-to-apical IVPG was significantly worse in groups A1 and A2 than in those in groups N1 and N2. On the other hand, basal IVPG was not significantly different between patients and normal controls (Fig. 2 and Supplemental Table 1).

Comparing the IVPGs between patients and normal controls based on age, the total, basal, and mid-to-apical IVPGs were not significantly different between younger and older subjects (Fig. 2 and Supplemental Table 1). The total, basal, and mid-to-apical IVPGs were not different between males and females in any of the 4 groups and neither between cancer diagnoses.

Correlation between IVPGs and conventional echocardiographic parameters

Table 3 shows the correlation between the IVPGs and conventional echocardiographic parameters. Non-normally distributed parameters were transformed to normal distribution by logarithmic transformation or Johnson normalization for calculating the correlation between IVPGs. Total, basal, and mid-to-apical IVPG were significantly correlated with e'. Concerning the correlation between IVPG and strain, both total and mid-to-apical IVPGs were significantly correlated with basal CS and papillary CS. Basal IVPG, however, was not correlated with any of these CS parameters except for basal CS. Although both total and basal IVPG were correlated with LS, mid-to-apical IVPG was not.

Correlation between the IVPGs and age-associated change and anthracycline use

Table 3 summarizes the correlation of IVPGs with age and anthracycline use. The total, basal, and mid-to-apical IVPGs were not significantly different based on either the age when the echocardiography performed, patient's age at the initiation of anthracycline therapy, duration after the initiation of anthracycline therapy, duration after the completion of anthracycline therapy, cumulative anthracycline dose, or cumulative radiation dose.

Reproducibility

Table 4 presents the results for intra- and interobserver variability. Important differences were not observed in the variability scores of the total, basal, and mid-to-apical IVPGs.

Discussion

Mechanisms of the total and mid-to-apical IVPG decreasing in childhood cancer survivors

Mid-to-apical IVPG is considered to be mainly affected by LV active suction and basal IVPG by LA pressure [21, 22], supported by animal experiment [23], although they cannot be divided clearly.

The major determinant of diastolic active suction is the elastic energy or elastic recoil, which is stored during systole; this is created by the fall of the LV volume under a critical value during contraction [13, 14]. This energy is promptly released during early diastole, causing active suction [13]. As the elastic recoil correlates with LV deformation in both the circumferential and longitudinal directions, strains may correlate with mid-to-apical IVPGs.

In our study, only basal CS decreased in all age groups and papillary CS decreased in the

older age groups, however apical CS and LS did not decrease at any age, these results basically corresponded our previously report [18]. Therefore, it is reasonable that mid-to-apical IVPG correlated only with basal and papillary CS, not with apical CS and LS. On the other hand, Iwano et al [22] showed significant correlation between mid-to-apical IVPD and not only papillary CS but also LS in subjects including patients with reduced both CS and LS. Importantly, CS showed a larger correlation coefficient with mid-to-apical IVPD compared to LS, which indicates that CS plays an important role in causing mid-to-apical IVPG.

The question remains whether basal CS affects basal IVPG or not. In our study, basal IVPG did not decrease in older patients, despite of the reduction of basal CS, which indicates decreased elastic recoil at basal segment. There is the possibility that basal CS weakly affected basal IVPG, basal CS weakly correlated with basal IVPG. However, as basal IVPG is mainly affected by LA pressure, not by elastic recoil [21, 22], it is reasonable that basal IVPG did not show a statistically significant difference between patients and controls. As E/e' in patients did not increase, they are thought to be at an early stage of diastolic dysfunction without elevation of LA pressure.

Furthermore, Cheung and Yu et al. [7, 24] demonstrated that the dyssynchronous motion of the LV is another mechanism of LV dysfunction with anthracycline therapy.

Dyssynchronous motion might have occurred in our patients, although we did not assess this factor. Because dyssynchronous motion of the LV causes impaired relaxation and an elongated tau index [25], it will inhibit LV suction.

In summary, the magnitude of decreases in the mid-to-apical IVPG may be caused by all of these factors. Therefore, the mid-to-apical IVPG could be the sensitive new indicator of cardiac diastolic dysfunction after the anthracycline therapy.

The time course of cardiac dysfunction and cardiac events

More than 15% of patients with anthracycline therapy had cardiovascular events during the long-term follow-up period, and the cumulative incidence of cardiovascular events continued to increase for more than 25 years [1]. Lipshultz et al. described the mechanisms of increased cardiac dysfunction and cardiac events in the long term [26]. The inhibition of myocardial growth by anthracycline is accentuated in children, whose LV mass is small. Although the increase of the LV mass is inhibited, afterload increasing becomes more obvious over time because of somatic growth. As a result, progressive cardiac abnormalities may occur [26]. In our study, the mean duration from chemotherapy to echocardiography was only 8.0 years. Therefore, regarding the time course, the measurements of conventional diastolic function did not show any

significant differences between patients and normal controls, despite the significant decrease of the mid-to-apical IVPG in patients of all ages and the total IVPG in the older age group, which indicate that the mid-to-apical IVPG will be sensitive indicator of cardiac dysfunction.

Clinical implications

Recently, Armstrong et al. [12] reported that the LS was a more sensitive indicator of cardiac dysfunction than the EF, because more than 30% of patients received anthracycline therapy had a reduced LS with a normal EF during long-term follow-up, with a median of 21 years from diagnosis. Furthermore, in a guideline, the use of LS was recommended to detect cardiac dysfunction in cancer survivors [27]. In our study, the mid-to-apical IVPG in patients of all ages decreased compared with that of normal controls, even though the conventional diastolic parameters, LVEF, and LS were not reduced. It was clearly too late to improve the cardiac function using cardioprotective agents such as β-blockers, angiotensin-converting-enzyme inhibitors, and angiotensin II receptor blockers in patients with a decreased EF [6, 9]. Importantly, although these cardioprotective agents effectively improved cardiac dysfunction in patients with a decreased LS and normal EF by anthracycline therapy, some patients did not respond to

these agents [9]. Therefore, the early detection of cardiac dysfunction due to anthracycline and cardioprotective treatment is important to improve the patient's prognosis. In our study, mid-to-apical IVPG was revealed as a sensitive new indicator and will be able to detect the subclinical cardiac dysfunction by anthracycline therapy earlier than that using conventional diastolic parameters or LS. It will allow us to provide earlier therapeutic intervention and will be able to improve the prognosis of childhood cancer survivors. Both these results and our previous study [18] demonstrated that basal CS in patients decreases from young age. Although currently it is not clear which of these factors is a more useful early indicator to detect cardiac dysfunction, both are significantly more useful than the other conventional echocardiographic parameters. To compare the clinical usefulness of IVPG and strains, we must observe long-term clinical outcomes using congestive heart failure, cardiac hospitalization, arrythmia, or cardiac mortality as the clinical goals or investigate the response to cardioprotective agents in the next study. However, although strains represent only the motion of ventricular segments, IVPG might be affected by all of the strains [22], dyssynchronous motion, [7, 24] and the speed of the ventricular motion at early diastole [16]; IVPG might represent total cardiac function. Therefore, IVPG may detect cardiac dysfunction with more sensitivity than strains.

Study Limitations

First, although we estimated the time course of the development of cardiac dysfunction in child cancer survivors, our study was a cross-sectional design. To know the true time course, a longitudinal follow-up study using a long duration is needed. The effect of the length of follow-up on cardiac growth and function may be clinically apparent only after prolonged observation about after 10-15 years from anthracycline therapy [1, 2, 26]. Second, the number of subjects was relatively small. Third, this study had the variation in types of cancers, the wide age range during chemotherapy, the varying duration after the completion of chemotherapy, and the cumulative anthracycline dose caused difficulty in analyzing the mechanisms of the reduced IVPGs. The use of other cytotoxic drugs and radiotherapy also affected the results [1, 12, 27]. It is very difficult to uniform all of these conditions. However, we firstly tested the IVPG to detect diastolic dysfunction with significant differences in childhood cancer survivors. Because our results are robust, we believe that our purpose was achieved in the study. In the future, more patient data should be collected to analyze the influence of each factor such as the age at the time of chemotherapy, age at echocardiography, duration since chemotherapy, types of disease, and radiation on the IVPGs. Furthermore, we must

observe long-term clinical outcomes or investigate the response to cardioprotective agents by using each of these echocardiographic parameters.

In conclusion, total IVPG in older cancer survivors and mid-to-apical IVPG in both younger and older cancer survivors were significantly and greatly reduced, although the conventional diastolic parameters and LS were normal. Consequently, mid-to-apical and total IVPGs are thought to be sensitive new indicators of diastolic cardiac function to detect early cardiotoxicity in survivors of childhood cancer.

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Figure legends

Fig. 1 a-e: Examples of the IVPG in a 14-year-old normal control. f-j: Examples of the IVPG in a 14-year-old survivor of childhood cancer with anthracycline therapy. Using a four-chamber view showing the mitral inflow, with the cursor parallel to the mitral

inflow (a, f), we captured the corresponding color M-mode Doppler image (b, g). The zero line of the Nyquist limit for two-dimensional color Doppler imaging was placed on the lower edge of the scale, and the Nyquist limit was set at 30% above the peak E wave velocity to mitigate the aliasing phenomenon (b, g). After the region of interest in the blue square box was determined (b, g), the program cord calculated the pressure gradient at each point. A three-dimensional temporal and spatial profile of the IVPG was generated (c, h). The x-axis represents the % time of systolic duration from 100% (at aortic valve closure) to end diastole. The y-axis represents the distance from the apex to the mitral valve in cm. The z-axis represents the IVPG in mmHg/cm. The peak IVPG in early diastole was identified (c, h). The red line (d, i) represents the temporal profile of the IVPG at the location of the peak IVPG. The x-axis represents the % time of systolic duration from aortic valve closure to end of the diastole, and the y-axis represents the IVPG in mmHg/cm. The red line (e, j) represents the spatial profile of the IVPG at the time of the peak IVPG. The x-axis represents the location from the apex to the mitral valve in cm, and the y-axis represents the IVPG in mmHg/cm. The total, basal, and mid-to-apical IVPG were automatically calculated. Survivors of a childhood cancer showed low total IVPGs with relatively larger basal IVPG and smaller mid-to-apical IVPG than normal controls. Even though the total IVPG profile of the normal

controls was larger than that in patients in this study, who had a relatively small basal IVPG and larger mid-to-apical IVPG.

IVPG: intraventricular pressure gradient; AoV: aortic valve; MV: mitral valve

Fig. 2 Box plots of the distribution of the IVPG values. Blue box: younger (age < 16 years) normal controls (Group N1). Green box: older (age ≧ 16 years) normal controls (Group N2). Yellow box: younger (age < 16 years) patients with anthracycline chemotherapy (Group A1). Red box: older (age ≧ 16 years) patients with anthracycline therapy (Group A2).

Total IVPG was significantly worse in subjects in group A2 than in those in group N2 (a). Mid-to-apical IVPG was significantly worse in groups A1 and A2 than in those in groups N1 and N2 (c). On the other hand, basal IVPG was not significantly different between patients and normal controls (b).

Comparing the IVPGs between young and old normal controls or patients, the total, basal, and mid-to-apical IVPGs were not significantly different between younger and older subjects (a, b, c).

*$p < 0.05$ refers to comparisons between normal controls and patients from the same age group (i.e., N1 vs. A1 and N2 vs. A2).

IVPG: intraventricular pressure gradient

Table 1 Characteristic of demographic data

	Young normal (N1)	Old normal (N2)	Young patients (A1)	Old patients (A2)
N (Female)	32 (20)	21 (9)	24 (16)	16 (8)
Age at echocardiography (years)	11.0 ± 2.5	22.2 ± 2.4††	11.9 ± 2.6	20.1 ± 2.8††
Weight (kg)	36.1 ± 11.2	61.8 ± 8.6††	39.6 ± 12.8	54.5 ± 11.8††
Height (cm)	143.6 (125.7, 153.3)	168.0 (160.5, 173.3) ††	145.6 (136.4, 154.4)	159.9 (153.6, 173.7) †
Body surface area (m^2)	1.18 ± 0.25	1.69 ± 0.16††	1.25 ± 0.26	1.53 ± 0.21†
Heart rate (bpm)	73.3 ± 12.6	62.4 ± 7.7†	71.2 ± 14.0	67.0 ± 10.1
Systolic blood pressure (mmHg)	104 ± 12	120 ± 11††	102 ± 9	106 ± 12*
Diastolic blood pressure (mmHg)	54.5 (49.3, 64.0)	71.0 (65.5, 77.5) ††	58.0 (55.5, 59.0)	58.5 (53.3, 63.8) **
Age at the initiation of anthracycline therapy (years)	-	-	5.3 (2.5, 8.4)	9.5 (4.4, 13.6) †
Duration after the initiation of anthracycline therapy (years)	-	-	8.3 (5.2, 11.3)	11.1 (8.2, 16.7) †
Duration after the completion of anthracycline therapy (years)			6.5 ± 3.3	10.2 ± 4.4 †
Cumulative anthracycline dose (mg/m^2)	-	-	147.2 ± 94.9	196.4 ± 146.5
<120 (n)	-	-	9	6
120-300 (n)	-	-	13	7

>300 (n)	-	-	2	3
Radiation (n)	-	-	4	5

*p < 0.05 and **p < 0.001 refer to comparisons between normal controls and patients and from the same age group; †p < 0.05 and ††p < 0.001 refer to comparisons between young and old normal controls or patients

Table 2 Echocardiographic measurements

	Young normal (N1)	Old normal (N2)	Young patients (A1)	Old patients (A2)	p (N1 vs. A1)	p (N2 vs. A2)	p (N1 vs. N2)	p (A1 vs. A2)
LVEF (%)	68.1 (64.3, 69.6)	60.8 (59.4, 65.5) ††	65.2 (64.2, 66.6)	63.1 (62.2, 65.6)	0.523	0.616	p < 0.001††	0.280
LVEDV/BSA (ml/m^2)	48.1 ± 14.2	49.7 ± 18.0	54.0 ± 18.1	55.3 ± 19.2	0.443	0.857	0.986	0.999
LVESV/BSA (ml/m^2)	16.2 ± 6.8	19.0 ± 7.4	18.9 ± 6.4	20.0 ± 7.6	0.493	0.976	0.482	0.962
E (m/sec)	1.05 ± 0.16	0.90 ± 0.11†	1.00 ± 0.17	0.86 ± 0.15†	0.714	0.826	0.003†	0.042†
A (m/sec)	0.48 ± 0.13	0.48 ± 0.10	0.48 ± 0.09	0.44 ± 0.11	0.999	0.757	1.000	0.781
E/A	2.24 ± 0.11	1.98 ± 0.14	2.21 ± 0.13	2.09 ± 0.16	0.999	0.950	0.467	0.933
e' (m/sec)	0.17 ± 0.03	0.16 ± 0.02	0.16 ± 0.03	0.15 ± 0.02	0.333	0.381	0.497	0.467
E/e'	6.19 ± 1.24	5.53 ± 0.82	6.32 ± 1.09	5.87 ± 0.92	0.968	0.773	0.134	0.563
IVRT (msec)	46.4 (31.5, 58.9)	64.0 (43.7, 72.2)	53.8 (40.7, 72.2)	64.6 (58.4, 75.4)	0.118	0.817	0.101	0.158
Basal CS (%)	-20.5 ± 3.1	-19.2 ± 2.8	-17.7 ± 3.5*	-15.0 ± 4.0*	0.015*	0.001*	0.520	0.057
Papillary CS (%)	-16.2 (-15.3, -18.9)	-17.2 (-15.6, -19.0)	-15.4 (-12.4, -18.8)	-15.4 (-13.1, -16.5) *	0.729	0.025*	0.755	0.934
Apical CS (%)	-22.9 ± 4.0	-21.3 ± 4.3	-21.0 ± 5.1	-20.4 ± 4.6	0.383	0.928	0.595	0.981
LS (%)	-17.7 ± 2.4	-17.1 ± 1.8	-16.5 ± 2.1	-17.1 ± 2.0	0.207	1.000	0.765	0.868

*p < 0.05 and **p < 0.001 refer to comparisons between normal controls and patients from the same age group; †p < 0.05 and ††p < 0.001 refer to comparisons between young

and old normal controls or patients
LV: left ventricle, LVEDV: left ventricular end-diastolic volume, LVESV: left ventricular end-systolic volume, BSA: body surface area, IVRT: isovolumic relaxation time, CS: circumferential strain, LS: longitudinal strain

Table 3 Correlation of IVPGs with LV conventional echocardiographic parameters and age and anthracycline use

	Total IVPG		Basal IVPG		Mid-to-apical IVPG	
	r	p	r	p	r	p
E	r = 0.458	< 0.001	r = 0.486	< 0.001	r = 0.129	0.033
e’	r = 0.393	< 0.001	r = 0.300	0.004	r = 0.327	0.001
E/e’ #	r = 0.086	0.417	r = 0.189	0.072	r = 0.073	0.492
Basal CS ##	r = -0.376	< 0.001	r = -0.228	0.030	r = -0.385	< 0.001
Papillary CS	r = -0.259	0.012	r = -0.160	0.125	r = -0.260	0.012
Apical CS	r = -0.073	0.487	r = -0.090	0.391	r = -0.020	0.848
LS	r = -0.216	0.037	r = -0.314	0.002	r = -0.105	0.997
Age at echocardiography (years)	r = -0.212	0.190	r = -0.248	0.122	r = -0.078	0.634
Age at the initiation of anthracycline therapy (years) #	r = -0.234	0.146	r = -0.235	0.144	r = -0.157	0.335
Duration after the initiation of anthracycline therapy (years)	r = -0.105	0.519	r = -0.104	0.525	r = -0.056	0.729
Duration after the completion of anthracycline therapy (years)	r = -0.125	0.442	r = -0.125	0.444	r = -0.067	0.682
Cumulative anthracycline dose (mg/m^2) #	r = -0.108	0.507	r = -0.101	0.536	r = -0.079	0.630

IVPG: intraventricular pressure gradient, CS: circumferential strain, LS: longitudinal strain

logarithmic transformation and ## Johnson normalization were performed these parameters from non-normal to a normal distribution. After these were normalized, we calculated the correlation between IVPGs and these parameters by parametric method using Pearson's correlation coefficient (r).

Table 4 Intra- and interobserver variability

	CV	ICC	Absolute difference/Mean (%)
Intraobserver variability in IVPG			
Total	3.90	0.984	3.47
Basal	8.28	0.942	6.18
Mid-to-apical	7.48	0.962	8.05
Interobsever variability in IVPG			
Total	4.81	0.968	4.31
Basal	8.55	0.929	8.45
Mid-apical	8.14	0.960	6.63

CV = Coefficient of variation, ICC = Intra class correlation

Supplemental table 1 IVPG measurements

	Young normal (N1)	Old normal (N2)	Young patients (A1)	Old patients (A2)
Total IVPG (mmHg/cm)	0.43 ± 0.09	0.39 ± 0.07	0.36 ± 0.12	0.29 ± 0.11*
Basal IVPG (mmHg/cm)	0.23 ± 0.06	0.18 ± 0.05	0.20 ± 0.09	0.15 ± 0.07
Mid-to-apical IVPG (mmHg/cm)	0.20 ± 0.05	0.21 ± 0.06	0.16 ± 0.05*	0.14 ± 0.06*

*$p < 0.05$ refers to comparisons between normal controls and patients from the same age group

IVPG: intraventricular pressure gradient

Figure 1

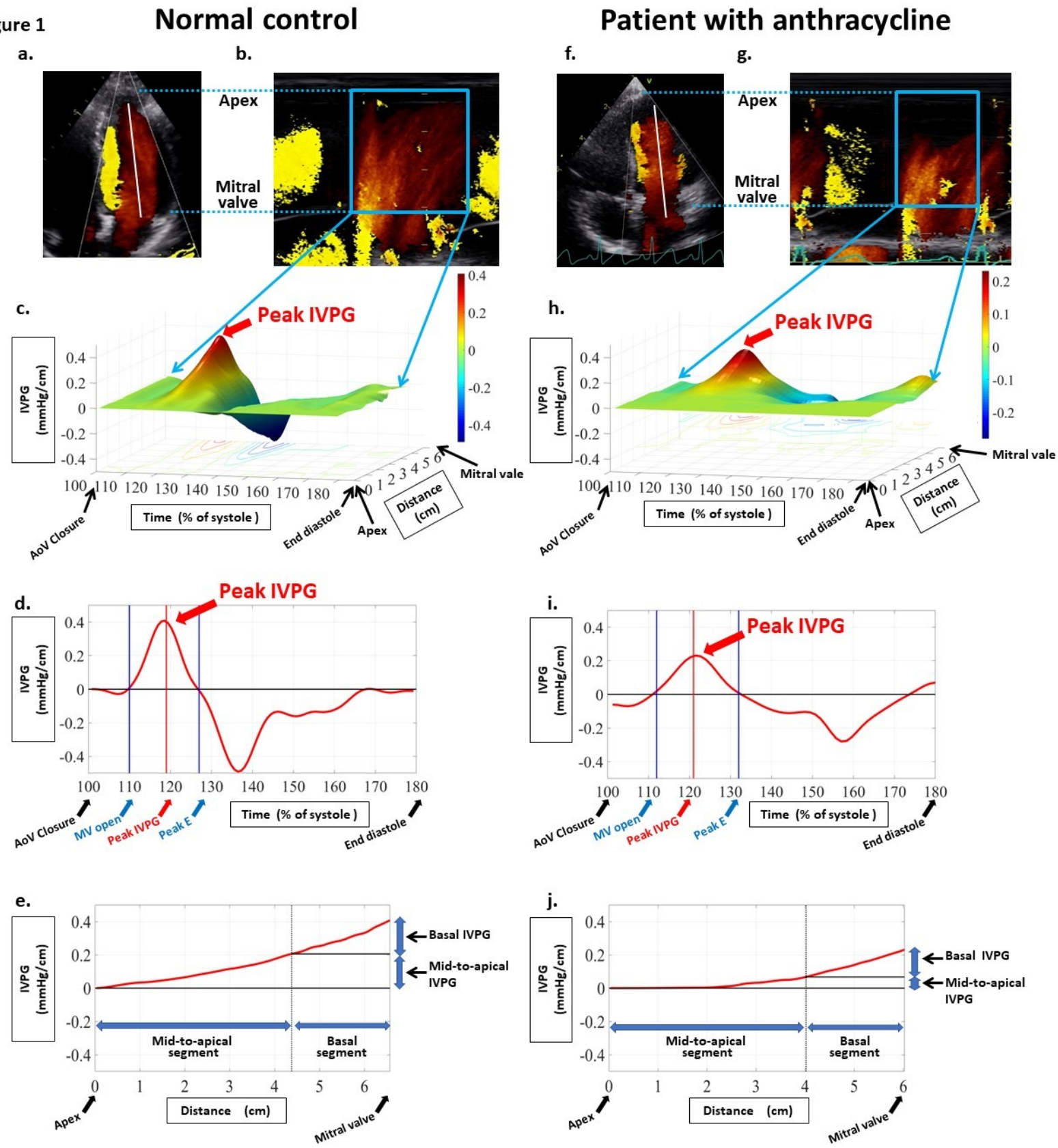
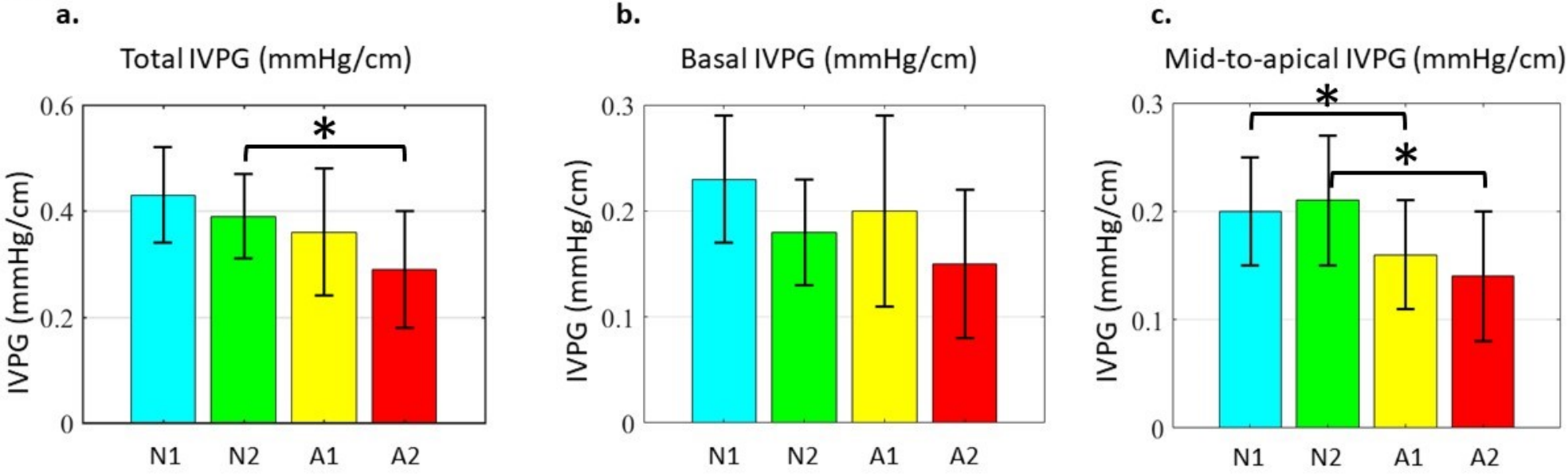

Normal control
Patient with anthracycline
a.
b.
f.
g.
Apex
Mitral valve
c.
h.
Peak IVPG
IVPG (mmHg/cm)
Time (% of systole)
AoV Closure
End diastole
Apex
Distance (cm)
Mitral vale
d.
i.
MV open
Peak IVPG
Peak E
e.
j.
Basal IVPG
Mid-to-apical IVPG
Mid-to-apical segment
Basal segment
Mitral valve

Figure2


a.
Total IVPG (mmHg/cm)
IVPG (mmHg/cm)
0.6
0.4
0.2
0
*
N1
N2
A1
A2
b.
Basal IVPG (mmHg/cm)
IVPG (mmHg/cm)
0.3
0.2
0.1
0
N1
N2
A1
A2
c.
Mid-to-apical IVPG (mmHg/cm)
IVPG (mmHg/cm)
0.3
0.2
0.1
0
*
*
N1
N2
A1
A2