Relationship between Coronary Microvascular Dysfunction and Left Ventricular Diastolic Function in Patients with Chest Pain and Unobstructed Coronary Arteries

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Abstract

Diastolic dysfunction (DD) is reported to affect up to 35% of the adult general population. The consequence of progressive DD is heart failure with preserved ejection fraction (HFpEF). Coronary microvascular dysfunction (CMD) has been suggested as one of the pathologic mechanisms leading to HFpEF. We investigated whether there was an association between coronary microvascular function and echocardiographic indices of left ventricular diastolic function in patients with chest pain and unobstructed coronary arteries (CPUCA). This retrospective observational study recruited patients referred to cardiology clinics assessment of chest pain who subsequently underwent assessment via CT coronary angiogram (CTA). Coronary microvascular dysfunction was determined by myocardial blood flow reserve (MBFR; <2.0) using myocardial contrast echocardiography. Echocardiographic indices of diastolic function (septal mitral annular e'; septal mitral annular E/e') were measured from baseline transthoracic echocardiogram. 149 patients (52% men) with a mean age 59.7(9.5) years were recruited. Mean (standard deviation) MBFR was 2.2 (0.51). 37% (55/149) had MBFR<2.0. Median [interquartile range] septal mitral annular e' velocity and septal mitral annular E/e' were 7.6 cm/s [6.2, 8.9] and 9.5 [7.5, 10.8] respectively. Univariate regression analysis showed only age was a significant predictor of increasing septal mitral annular E/e' (?=+0.20 95% CI 0.13, +0.28, p<0.001) but not MBFR. Multivariable analysis also showed no association between these septal mitral annular E/e' and MBFR after adjustment for cardiovascular risk factors. There was no relationship found between echocardiographic indices of left ventricular diastolic function and coronary microvascular function.

INTRODUCTION

There is an increasing body of evidence linking coronary microvascular dysfunction (CMD) with left ventricular (LV) diastolic dysfunction and a future risk of developing heart failure (HF) with preserved ejection fraction (HFpEF).[1–3] This is of clinical significance as it has been recently shown that patients with CMD-associated diastolic dysfunction have an up to three-fold increased mortality rate and up to five-fold risk of developing a major adverse cardiovascular event (MACE).[4] Previous studies have also reported similar changes in the echocardiographic indices of diastolic dysfunction in patients with CMD including reduction in left ventricular (LV) early diastolic strain rate, early volumetric filling rate and elevated LV filling pressures.[5,6] However, there is a relatively limited amount of published research in this area regarding the relationship between CMD and LV diastolic function. A better understanding could help target future ther-

apies against this condition. This study sought to investigate whether there was an association between CMD and LV diastolic function in a group of patients presenting with CPUCA.

METHODS

Study design

This was a single centre, prospective cross-sectional, observational study carried out at Poole Hospital NHS Foundation Trust, UK. The study recruited patients between 2011-2013 aged 30-80 years who were referred to the cardiology clinics with chest pain suggestive of myocardial ischemia and subsequently had a diagnostic CT coronary angiography (CTA) demonstrating no evidence of significant coronary stenosis. Exclusion criteria were known ischemic heart disease (previous acute coronary syndrome, previous percutaneous coronary intervention or previous coronary artery bypass graft), valvular heart disease, LV hypertrophy or LV ejection fraction <55%. We obtained information regarding patient baseline demographics including age, body mass index (BMI), hypertension, smoking status, dyslipidemia and presence or absence of diabetes. Smoking status included both current and past smokers. Blood samples were analyzed for fasting lipid profile (low density lipoprotein (LDL) and triglyceride (TG)). Given the possible association between HFpEF and inflammation,[7] levels of high sensitivity C-reactive protein (Hs-CRP), a marker of systemic inflammation, were also measured in our patients.

CT-Coronary Angiogram

CT coronary angiogram (CTA) was performed using a 64-channel CT scanner (GE Lightspeed VCT, GE Medical Systems). Reconstructed CTA images were analyzed on a dedicated 3-dimensional workstation (CardIQ Xpress, GE Medical Systems) with curved multiplanar reformation and short-axis cross-sectional viewing techniques. The mean heart rate during the scan was 64 beats/min. Calcium scoring and helical scan data was performed on all patients using prospective ECG triggering at 75% of the R-R interval. Following this, a 20ml bolus of contrast (Niopram 370®) at 6mls/sec was injected and the timing of peak contrast enhancement in the aortic arch was used to determine the timing of scan acquisition. The contrast-enhanced scan used 80mls contrast injected at 6 ml/s, followed by a 50ml at 6mls/sec saline flush, during a single expiration breath hold. The CTA scan parameters were: collimator 20 mm, slice thickness 0.625 mm; gantry rotation 350 ms; helical acquisition using a pitch of 0.16; tube current 455–515 mA with ECG tube current modulation; tube voltage range 100–140 kV; rotation time, 350 ms. The estimated radiation dose per patient was 3.3 mSv. Unobstructed coronary artery disease was defined as a quantitatively measured luminal stenosis<50%. All patients were in sinus rhythm.

Baseline Echocardiography and Diastolic Function Assessment

All patients underwent resting transthoracic echocardiography (TTE) for analysis of LV function, assessment of LV diastolic function and exclusion of existing valvular pathologies using standard echocardiographic views. Early diastolic transmitral filling velocity (E) was measured at the mitral valve leaflets tips using pulsed-wave doppler while spectral tissue doppler imaging was used to measure early diastolic relaxation velocities (e') at the septal mitral annulus from the apical four-chamber window as previously described.[8] LV diastolic function was measured using septal mitral annular E/e' ratio (the ratio between early mitral inflow velocity and mitral annular early diastolic velocity). Elevated LV filling pressures were defined as septal mitral annular E/e' ratio [?] 15.[8]

Coronary Microvascular Function assessment

Noninvasive quantification of coronary microvascular function was performed using myocardial contrast echocardiography (MCE).[9] This has previously been shown to correlate with PET derived myocardial blood flow.[10] Briefly, MCE was performed using ultrasound machine iE33 (Phillips Medical Systems) and SonoVue (Bracco Research SA) as the contrast infusion given at constant infusion. Real time echocardiographic images were recorded within 3-4 minutes in the apical 4, 2, and 3-chamber views with low-power settings at mechanical index of 0.1. SonoVue was at an infusion rate of 60 ml/h via peripheral intravenous access with VueJect infusion syringe pump (Bracco Research, SA). Thereafter, rate was maintained between 48

and 60 ml/h to maximise image quality. Machine settings were held constant throughout each participants study post optimisation. Flash-impulse imaging using high mechanical index of 1.0 was performed to achieve complete myocardial bubble destruction, after which 10 end-systolic frames were recorded in each apical view. Dipyridamole was infused at 0.56 mg/kg over a 4-minute period after acquisition of resting images. Post stress images were recorded within 3 to 4 minutes after an interval of 2 minutes. Quantitative analysis was performed offline using QLab V7.0 (Philips Medical Systems) blinded to patient demographic and CTCA data. Quantitative assessment of myocardial perfusion was performed for 10 consecutive end-systolic frames after microbubble destruction. A region of interest was placed over the thickness of the myocardium. Plots of peak myocardial contrast intensity (linearly related to myocardial blood volume A cm^3) versus pulsing intervals (representing time) were automatically constructed to fit the mono-exponential growth function: $y=A\ (1 - e^{-Bt})$ where B is the instantaneous initial slope of the resulting curve and represents myocardial blood velocity (sec^{-1}) and the product of A and B yields a reliable measure of myocardial blood flow (MBF) ($cm^3.sec^{-1}$).

MBFR was measured as previously defined as the ratio of post-dipyridamole (stress) MBF to baseline MBF, dividing the stress MBF by the baseline MBF for the same segment.[9] A 16-segment model was used excluding the basal segments in view of contrast attenuation and analyzing the 10 remaining mid- and apical cardiac segments. A segment was excluded if there was artefact, inadequate microbubble destruction, attenuation or a wide variation in contrast intensity. Segmental MBFR was calculated by dividing peak MBF with resting MBF of the same segment. MBFR was the average MBFR of all segments. CMD was defined as MBFR<2.0.[9]

Statistical analysis

Normality of continuous variables was quantified using the Shapiro-Wilk test and was reported as the mean (standard deviation, SD) and the median [inter-quartile range, IQR] for parametric and non-parametric data respectively. Two group comparisons of continuous data for those with and without CMD was performed using a non-paired t-test and Wilcoxon rank-sum tests respectively. Comparisons between the MBFR < 2.0 group and the MBFR [?] 2.0 group were undertaken using a Fisher's Exact Test.

In univariate and multivariate analysis, septal mitral annular E/e' was used as the main outcome variable while known influencers of septal mitral annular E/e' (age, gender, diabetes, hypertension, smoking status, Body Mass Index (BMI), fasting TG, fasting LDL, MBFR and Hs-CRP) were used as the input variables. For multivariate analysis, an initial fully saturated model was constructed using all the known predictor variables. Non-significant predictors were sequentially deleted, and the model re-run. Likelihood ratio testing was employed to quantify significant differences, or lack thereof, between model iterations. The final models only contained the significant predictors. Results are reported as regression slope (β) and its level of significance. Where required, inferential analysis was performed using standard regression techniques except in the case of a dichotomous outcome variable, where logistic regression was employed. Predictor variables were tested for co-linearity using a standard Pearson's correlation. Variables with correlation coefficients (R) greater than ± 0.80 were considered co-linear with one or other excluded largely on empirical grounds. Where appropriate, dummy variables were used when examining the significance of categorial predictors and all two-way interactions were tested for significance. Finally, all statistical models were tested for adequacy using appropriate regression diagnostics with regression residuals tested for normality using the Shapiro-Wilk test and for homoscedascity using the Breusch-Pagan test.

Statistical analysis was performed using a propriety statistical package (STATA version 15.1) with the level of significance set at $p < 0.05$.

Ethics

This study complied with the Declaration of Helsinki and was approved by the local research ethics committee (H0102/78). All patients provided a written informed consent.

RESULTS

Baseline participant characteristics

The baseline characteristics for all patients are as listed in Table I. We recruited 183 patients of whom 34 studies had to be subsequently excluded from the analysis due to poor echocardiographic image quality (Figure I). No significant differences in patient demographics were found between those included in the analysis and those excluded from the study. The mean age of the study population was 59.7(9.5) years of whom 52% were male, 11% were diabetics, 56% had dyslipidemia and 39% had hypertension. The measured median septal mitral annular e' was 7.6 cm/s [6.2, 8.9] while the measured median septal mitral annular E/e' was 9.5 [7.5, 10.8]. In addition, the measured mean Hs-CRP was 2.6 [0.7, 3.0]. Elevated LV filling pressures (septal mitral annular E/e' ratio [?] 15) were present in a total of 10 (6.7%) patients. Five out of the 10 patients with elevated LV filling pressures had evidence of CMD. Only age was the significant predictor for elevated LV filling pressures (Table II) with an odds ratio of 1.07 (95% CI [1.03, 1.13], p=0.001).

Characteristics of patients with coronary microvascular dysfunction

CMD was present in 55 (37%) patients with Table I showing baseline characteristics of patients subdivided into those with and without CMD. There were more patients with diabetes, hypertension, and higher LDL levels in those with CMD compared to those without. However, there were no significant differences between septal mitral annular e' (cm/s) or septal mitral annular E/e' ratio in both groups. Hs-CRP levels were also similar in both groups.

Relationship between coronary microvascular dysfunction and left ventricular diastolic function

In a univariate regression analysis using MBFR as a continuous independent variable and septal mitral annular E/e' as a continuous outcome variable (Table III), no significant association was found between MBFR and septal mitral annular E/e' (β=-0.24 95% CI -1.53, +1.05, p=0.71). Increasing age was the only significant predictor for increasing septal mitral annular E/e' (β=+0.19 95% CI +0.12, +0.25, p=<0.001). Multivariable regression analysis in a model using known influencers of LV diastolic function (age, sex, diabetes, hypertension, smoking status, BMI, fasting TG, fasting LDL, MBFR and Hs-CRP) showed only age was the significant predictor of increasing septal mitral E/e' (β=+0.20 95% CI +0.13, +0.28, p<0.001, r^2=0.21) (Table IV).

Sensitivity analysis of the model above was performed instead defining CMD as a binary independent variable (MBFR<2.0) and septal mitral annular E/e' as a continuous outcome variable. No significant association was found between patients with evidence of CMD with increasing septal mitral annular E/e' in the univariate (β=-0.57, p=0.8) or multivariable regression analysis.

DISCUSSION

This study evaluated the association between MBFR, a measure of coronary microvascular function and LV diastolic function, measured via septal mitral annular E/e' in a group of patients presenting with chest pain and unobstructed coronary arteries. Our study showed that there was no association found between MBFR and LV diastolic function in both univariate and multivariate regression analysis after taking traditional cardiovascular risk factors into account.

Contrary to the findings from Taqueti,[4] we did not find any correlation between septal mitral annular E/e' and MBFR. In their study, they included patients with a similar profile (patients presenting with chest pain and no evidence of flow limiting coronary artery disease (CAD) on PET) yet found that impaired coronary flow reserve (CFR) in these patients was independently associated with diastolic dysfunction.[4] In addition, they also used a similar echocardiographic definition for LV diastolic dysfunction to our study (E/e' septal>15) and demonstrated a significant direct relationship between CFR and septal e' and an inverse relationship between CFR and E/e' septal. Their findings were clinically important as those patients with evidence of both CMD and elevated septal E/e' ratio had a five-fold increased risk for HFpEF hospitalisation after a median follow up of 4 years.[4]In our study, only 5 out of the 55 (9%) patients with CMD had evidence of raised LV filling pressures. It is not clear from the study by Taqueti how many patients with CMD also had

echocardiographic evidence of elevated LV filling pressures (septal mitral annular E/e' ratio [?] 15), although the published median and quartiles for E/e' were 13.0 (9.3-16.1) in patients with CMD vs 10.8 (8.7-12.8) in patients without CMD.[4]

A larger prospective multicenter study by Shah enrolled 202 patients with a confirmed diagnosis of HFpEF and measured the prevalence of CMD via left anterior descending artery Doppler flow signals at rest and during adenosine infusion.[11] CMD was present in nearly 75% of patients and associated with abnormal longitudinal myocardial function assessed by TTE. However, the main limitation of their study was the lack of exclusion of macrovascular CAD which would have led to a lower measurement of CFR. [11,12]

There are several potential reasons that might explain the discrepancy between the findings in our study and previous studies. Firstly, in the study by Taqueti, their patient cohort included a greater proportion of comorbidities known to affect both coronary microvascular function and myocardial stiffness.[4] The difference between their study population and ours were the higher numbers of the patients who were elderly (median age of 66 vs 59.7), proportions female (64.7% vs 48%), hypertensive (75.6% vs 39%) and had diabetes mellitus (32.8% vs 11%).[4] Additionally, it is possible that despite the presence of CMD, our patient cohort with its lower prevalence of co-morbidities, may be earlier on the spectrum of myocardial stiffness and not yet manifesting detectable diastolic dysfunction.[11,12]

Notably, approximately 25% of patients who met the criteria for HFpEF in the study by Shah did not have evidence of CMD.[11]This is interesting as it could be explained by the heterogenous nature of HFpEF driven by non-cardiac specific causes of fluid overload, and that besides endothelial dysfunction, there may be other factors causing HFpEF syndrome in these patients.[11] This implies that the presence of CMD does not necessarily equate to the reduction of diastolic function, hence the absence of correlation between CMD and LV diastolic function in our study.

The main question of the causal mechanistic links of whether 1) CMD leads to LV diastolic dysfunction and subsequently HFpEF or 2) LV diastolic dysfunction observed in patients with HFpEF leads to CMD, remains unclear. As alluded to, it is possible that our patient cohort is at an early stage of CMD, prior to the development of diffuse myocardial fibrosis and subsequent increase in LV stiffness via recurrent micro-infarctions secondary to coronary microvascular ischaemia.[13] This hypothesis is supported by the finding of an exacerbated diastolic dysfunction when there was a detectable troponin in Taqueti's study.[4,13] Limited by the retrospective nature of this study, it would be interesting to observe prospectively how many of our patients with CMD eventually developed evidence of LV diastolic dysfunction or HFpEF.

Previous studies have also postulated that CMD is a consequence of a systemic pro-inflammatory state which occurs in medical conditions such as diabetes or obesity.[1,6,7,14] Higher levels of Hs-CRP have also been observed in patients with HFpEF than in patients with heart failure with reduced ejection fraction (HFrEF), supporting a link between inflammation and HFpEF.[7] However, in our study, we did not find an association between reduced MBFR and raised Hs-CRP. This potentially is a result of a lower prevalence of medical co-morbidities in the patients recruited in our study.

Study limitations

There are several limitations to our study. Firstly, this was a single center study which increases the potential for selection bias. There were also several patients excluded due to inadequate echocardiographic image quality which could have introduced an additional bias. However, no significant differences in patients baseline demographics were found between those included and excluded from this study. The utilization of only septal e' and septal mitral annular E/e' ratio is also a limitation to the assessment of LV diastolic function, as this is susceptible to changes in LV loading conditions. Nonetheless, these were the same echocardiographic parameters used in previous studies. Ideally, a more recent criteria to measure diastolic function should be applied but we were limited to the data available.

Conclusions

In conclusion, this study did not find an association between CMD and echocardiographic indices of LV diastolic function in patients presenting with chest pain and unobstructed coronary arteries.

Table I: Baseline participant demographics grouped according to the presence (MBFR <2.0) or absence (MBFR >2.0) of coronary microvascular dysfunction**.** Values are given as mean (SD), median [IQR] or n (%). P-value denotes differences between groups.

Variable	Overall (n=149)	MBFR <2.0 (n=55)	MBFR [?]2.0 (n=94)	P-value
Age (years)	59.7 (9.5)	61 (8.6)	59 (9.8)	0.15
Male sex	78 (52%)	29 (53%)	49 (52%)	0.99
Diabetes	16 (11%)	11 (20%)	5 (5%)	0.02
BMI (kg/m^2)	27.3 (3.6)	28.4 (3.3)	26.9 (3.7)	0.052
Smoking history	70 (47%)	30 (55%)	40 (43%)	0.18
Hypertension	58 (39%)	28 (51%)	30 (32%)	0.025
Dyslipidemia	84 (56%)	34 (62%)	50 (53%)	0.41
Total cholesterol (mmol/L)	5.2 (1.1)	5.0 (1.2)	5.4 (1.0)	0.07
Triglycerides (mg/dL)	1.6 (1.3)	1.7 (1.1)	1.6 (1.4)	0.66
Hs-CRP (units)	1.4 [0.7, 3.0]	2.1 [0.8, 3.6]	1.3 [0.7, 3.0]	0.13
Septal mitral annular e' (cm/s)	66 [56, 78]	70 [58, 83]	65 [56, 77]	0.32
Septal mitral annular E/e' ratio	13.7 [11.2, 16.1]	13.9 [11.1, 15.6]	13.6 [11.2, 16.5]	0.71

Table II: Multivariate regression modelling of demographic variables, lipid profile, coronary microvascular function and inflammatory markers against septal mitral annular E/e' [?] 15 as a dichotomous outcome variable.

Variable	**Odds ratio**	**95%CI**	**P-value**
MBFR	1.19	0.55, 2.57	0.67
Age (years)	1.08	1.03, 1.13	0.002
Male sex	1.16	0.52, 2.58	0.72
Diabetes	1.78	0.51, 6.23	0.36
Hypertension	0.89	0.39, 2.05	0.79
BMI (kg/m^2)	1.10	0.97, 1.26	0.13
LDL (mmol/L)	1.23	0.79, 1.92	0.36
Hs-CRP (units)	0.98	0.87, 1.10	0.76

Table III: Univariate regression modelling of demographic variables, lipid profile, coronary microvascular function and inflammatory markers against septal mitral annular E/e' as a continuous outcome variable.

Variable	**Υνιαριατε μοδελ β**	**95% CI**	**P-value**
MBFR	-0.24	-1.53, +1.05	0.71
Age (years)	+0.19	+0.12, +0.25	<0.001
Male sex	-0.77	-2.01, +0.53	0.24
Diabetes	+0.85	-1.18, +2.89	0.41
Hypertension	+0.38	-0.95, +1.71	0.58

Variable	Υνιαριατε μοδελ β	95% CI	P-value
BMI (kg/m²)	+0.07	-0.12, +0.26	0.46
LDL (mmol/L)	+0.47	-0.23, +1.18	0.19
Hs-CRP (units)	-0.07	-0.24, +0.09	0.36

Table IV: Multivariable regression analysis in a model using known influencers of LV diastolic function.

Variable	β (95% ˜I)	p-value
Age	+0.20 (+0.13, +0.28)	<0.001
Sex	-0.28 (-1.68, +1.12)	0.69
Diabetes	+0.43 (-1.96, +2.82)	0.72
Hypertension	-0.06 (-1.55, +1.44)	0.94
Smoking status	+0.27 (-0.90, +1.44)	0.65
BMI	+0.08 (-0.14, +0.30)	0.47
Triglycerides	+0.52 (-0.57, +1.61)	0.35
LDL	+0.41 (-0.38, +1.21)	0.31
MBFR	+0.30 (-1.11, +1.71)	0.67
Hs-CRP	-0.10 (-0.26, +0.06)	0.22

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Figure I: Patient flow diagram.