Associations of Macro- and Microvascular Endothelial Dysfunction With Subclinical Ventricular Dysfunction in End-Stage Renal Disease

Ruth F. Dubin, Isabella Guajardo, Amrita Ayer, Claire Mills, Catherine Donovan, Lauren Beussink, Rebecca Scherzer, Peter Ganz, Sanjiv J. Shah

Abstract—Patients with end-stage renal disease (ESRD) suffer high rates of heart failure and cardiovascular mortality, and we lack a thorough understanding of what, if any, modifiable factors contribute to cardiac dysfunction in these high-risk patients. To evaluate endothelial function as a potentially modifiable cause of cardiac dysfunction in ESRD, we investigated cross-sectional associations of macro- and microvascular dysfunction with left and right ventricular dysfunction in a well-controlled ESRD cohort. We performed comprehensive echocardiography, including tissue Doppler imaging and speckle-tracking echocardiography of the left and right ventricle, in 149 ESRD patients enrolled in an ongoing prospective, observational study. Of these participants, 123 also underwent endothelial-dependent flow-mediated dilation of the brachial artery (macrovascular function). Microvascular function was measured as the velocity time integral of hyperemic blood flow after cuff deflation. Impaired flow-mediated dilation was associated with higher left ventricular mass, independently of age and blood pressure: per 2-fold lower flow-mediated dilation, left ventricular mass was 4.1% higher (95% confidence interval, 0.49–7.7; \( P = 0.03 \)). After adjustment for demographics, blood pressure, comorbidities, and medications, a 2-fold lower velocity time integral was associated with 9.5% higher E/e′ ratio (95% confidence interval, 1.0–16; \( P = 0.03 \)) and 6.7% lower absolute right ventricular longitudinal strain (95% confidence interval, 2.0–12; \( P = 0.003 \)). Endothelial dysfunction is a major correlate of cardiac dysfunction in ESRD, particularly diastolic and right ventricular dysfunction, in patients whose volume status is well controlled. Future investigations are needed to determine whether therapies targeting the vascular endothelium could improve cardiac outcomes in ESRD. (Hypertension. 2016;68:00-00. DOI: 10.1161/HYPERTENSIONAHA.116.07489.) • Online Data Supplement

Key Words: cardiovascular disease • echocardiography • end-stage renal disease • heart failure

Cardiovascular disease (CVD) accounts for nearly half of the high mortality rate among patients with end-stage renal disease (ESRD); the prevalence of atherosclerotic disease and heart failure are 70% and 45%, respectively, for patients on hemodialysis.1 Unfortunately, we lack effective cardiovascular therapies for these patients, in part because of an incomplete understanding of the cardiovascular physiology afflicting this population. Aside from volume overload, we do not know whether modifiable factors, such as endothelial dysfunction, contribute to cardiac dysfunction in these patients. Among patients with chronic kidney disease (CKD), endothelial dysfunction is common2,3 and predicts adverse outcomes.4,5 A previous study did not find an association between endothelial dysfunction and cardiac mechanics in patients with ESRD,6 but we hypothesized that such an association could be found with more sensitive measures of cardiovascular dysfunction and consistent timing of cardiovascular measurements with respect to dialysis. The CERES study (Cardiac, Endothelial Function and Arterial Stiffness in ESRD) is an ongoing, prospective, observational study designed to provide in-depth measures of vascular and cardiac function and determine the use of these measures in predicting heart failure and overall mortality in ESRD.

Endothelium-dependent, flow-mediated dilation (FMD) of the brachial artery is a gold-standard, noninvasive method of quantifying endothelial NO bioavailability7 that correlates with coronary endothelial function.8 FMD, the percent change in diameter of the brachial artery during hyperemic blood flow, represents macrovascular endothelial function, and velocity time integral (VTI) of hyperemic blood flow is considered a marker of microvascular endothelial function.9 Speckle-tracking echocardiography is becoming a widely accepted, state-of-the-art research tool.10,11 Systolic function, measured using speckle-tracking echocardiography as global longitudinal strain, is associated with adverse outcomes in patients with12,13 and without CKD.14 Tissue Doppler imaging,
summarized by $E/e'$ as a measure of diastolic dysfunction and loading conditions, predicts adverse outcomes in ESRD.\textsuperscript{15,16}

We sought to delineate associations between endothelial function and cardiac mechanics in ESRD, using baseline FMD and cardiac measures in CERES participants. We hypothesized that advanced methods, consistently timed with respect to dialysis, would demonstrate associations between endothelial function and cardiac mechanics in ESRD patients without symptoms of heart failure, supporting future research into endothelial health-promoting therapies to prevent heart failure and other adverse cardiovascular outcomes in these patients.

**Methods**

**Study Population**

The CERES study is an ongoing, prospective observational study designed to obtain high-quality echocardiography and vascular measures performed at consistent intervals with dialysis and to follow longitudinal outcomes in a cohort of well-controlled hemodialysis patients. Patients were recruited by mail and phone from the San Francisco Bay Area kidney transplant waitlist, the San Francisco General Hospital Chronic Dialysis Unit and 3 local Fresenius dialysis units. To be included, patients had to be on hemodialysis or peritoneal dialysis. Any of the following led to exclusion: more than moderate valvular disease, recent myocardial infarction or major surgery, current infection, newly diagnosed or metastatic cancer, cocaine or intravenous drug use. Patients who had undergone surgical procedures in both arms were excluded from having vascular studies. Study visits took place at the San Francisco General Hospital Clinical Research Center. Medical history, such as CVD, cause of renal disease, and pack-years of smoking tobacco, was obtained by self-report. Information such as medications and monthly laboratory tests was obtained from the participants’ dialysis units. The study was performed according to the principles of the Declaration of Helsinki. Patients gave written informed consent, and the study protocol was approved by the University of California, San Francisco Committee for Human Research.

Methods for endothelium-dependent FMD and echocardiography are given in the online-only Data Supplement.

**Statistical Analysis**

First, we analyzed demographic and clinical characteristics of all participants whose echocardiograms were included in the analyses. We also examined differences in demographics and comorbidities between subgroups (eg, those that could or could not undergo FMD and those above or below median FMD and VTI.) Next, we used Spearman correlations to examine unadjusted associations of FMD and VTI with parameters of left ventricular (LV) and right ventricular (RV) mechanics. For LV systolic function, we relied primarily on longitudinal strain (by review of the Bay Area Transplant Waitlist); 32% self-reported atherosclerotic disease (defined as myocardial infarction, stroke, peripheral artery disease, percutaneous coronary intervention, or coronary artery bypass graft). SBP was well controlled, and hemoglobin levels measured on the day of study visit (the day after dialysis) were higher than those taken from patients’ monthly laboratory tests (normally drawn immediately predialysis): 11.4±1.3 versus 10.8±1.1 g/dL ($P<0.001$). Because fluid removal during dialysis results in an increase in hemoglobin, higher hemoglobin at study visit supports that most patients were euolemic. Mean LV mass index was 118 (±31) g/m², and the majority had ejection fraction >50% (Table 2).

**Reproducibility**

Eleven patients returned for repeat studies performed 7 days after the initial visit, on the day after dialysis. Mean (SD) absolute difference in FMD and VTI between study visits were as follows: FMD 1.6% (±1.4) and VTI 15 cm (±13).

**Associations of Vascular and Cardiac Parameters**

In unadjusted analyses, we found that higher FMD (better endothelial function) correlated with lower LV mass and lower $E/e'$ ratio (better diastolic function) and with higher RV early diastolic strain rate (better diastolic function). By comparison, higher VTI (better endothelial function) correlated with lower $E/e'$ and with higher RV free wall systolic strain (better systolic function). Correlations of FMD and VTI with other left and right ventricular parameters were weaker and did not reach statistical significance (Figure and Table 3).

We then performed multivariable linear regression analysis to determine whether the associations of FMD and VTI with LV parameters were independent of demographic factors,

All statistical analyses were performed with STATA statistical analysis software version 11.0 (College Station, TX).

**Results**

Characteristics of the cohort are displayed in Table 1. The mean±SD age was 55±13 years, 114 (77%) were of nonwhite race. Patients were relatively healthy for an ESRD cohort: 29% were actively listed for transplant at the time of recruitment (by review of the Bay Area Transplant Waitlist); 32% self-reported atherosclerotic disease (defined as myocardial infarction, stroke, peripheral artery disease, percutaneous coronary intervention, or coronary artery bypass graft). SBP was well controlled, and hemoglobin levels measured on the day of study visit (the day after dialysis) were higher than those taken from patients’ monthly laboratory tests (normally drawn immediately predialysis): 11.4±1.3 versus 10.8±1.1 g/dL ($P<0.001$). Because fluid removal during dialysis results in an increase in hemoglobin, higher hemoglobin at study visit supports that most patients were euolemic. Mean LV mass index was 118 (±31) g/m², and the majority had ejection fraction >50% (Table 2). The median FMD was 4.3% (interquartile range, 2.5–6.9) and median VTI was 67 cm (interquartile range, 47–81). Participants with VTI below the median tended to be older, to be of black race, and to have a history of atherosclerotic disease. Those with FMD below the median tended to self-report diabetes mellitus as the cause of their renal disease, to be of black race, and to have higher SBP and body mass index (online-only Data Supplement). Patients who did not undergo FMD testing because of having had bilateral upper extremity surgical interventions (n=20) tended to have been on dialysis longer (median, 79 versus 46 months; $P=0.003$) and were less likely to be actively listed for kidney transplant (33% versus 55%; $P=0.006$).
comorbidities, and cardiovascular medication use. Higher FMD remained associated with lower LV mass after adjustment for age and SBP, but addition of demographics (particularly sex) rendered the association statistically nonsignificant. Higher VTI remained significantly associated with lower E/e’ ratio, even after adjusting for medications (−9.5% per doubling of VTI, 95% confidence interval, −16 to −1.0; Table 4).

We performed similar multivariable linear regression analysis of the associations of endothelial and RV parameters. Higher
VTI remained associated with higher RV systolic free wall strain after multivariable adjustment (6.7% per VTI doubling, 95% confidence interval, 2.0–11.6). Likewise, higher VTI remained associated with higher RV early diastolic strain rate (11.6% per VTI doubling, 95% confidence interval, 3.0–21; Table 5).

We compared endothelial function among patients with diastolic dysfunction categorized as normal, mild, moderate, and severe diastolic dysfunction. Mean (SD) FMD were 5.7 (±3.9), 4.8 (±3.3), 4.4 (±2.9), and 3.5 (±1.6; ANOVA P=0.34, P for trend=0.14); VTI were 72 (±28), 68 (±35), 53 (±27), and 48 (±30; ANOVA P=0.08, P for trend=0.03). These results are in agreement with the correlation of VTI and diastolic dysfunction, measured as E/e′. In a sensitivity analysis of a subset of 83 patients who did not self-report atherosclerotic disease, the only correlation that remained significant (P<0.05) was VTI and RV free wall systolic strain (R=−0.22; P=0.05). The correlation of FMD and LV mass became less significant (R=−0.21; P=0.09). The correlation of VTI and LV mass remained statistically insignificant but became a trend (R=0.19; P=0.09).

Discussion

We present several salient findings that, to our knowledge, have not been previously demonstrated in the setting of ESRD or other populations. Namely, microvascular endothelial dysfunction (VTI) has an independent association with LV diastolic dysfunction, RV systolic dysfunction, and RV diastolic dysfunction. Macrovascular function was associated with LV mass, but after full adjustment, the association was not significant. For this discussion, it is important to note that brachial FMD and VTI are considered important correlates of cardiac endothelial function.

Association of Macrovascular Function (FMD) With LV Mass

In animal knockout models, mice deficient in endothelial or neuronal NO synthases rapidly develop LV hypertrophy.17 In ESRD, high levels of asymmetrical dimethylarginine inhibit NO synthases, mimicking the knockout state.18 There have been 2 large studies showing associations between FMD and LV mass in community-dwelling populations,19,20 and smaller studies have demonstrated this association in hypertensive patients.21–23 There have been relatively few studies in patients with CKD. Our findings are in accordance with a previous study by Pannier et al24 showing that forearm reactive hyperemia, measured by venous plethysmography, correlated with LV mass index and common carotid media–intima thickness. However, a previous study by Fathi et al did not find correlations between FMD and cardiac structure and function. In the subgroup without self-reported CVD, the microvascular function–LV mass association gained statistical significance. As a potential explanation for this observation, microvascular
dysfunction may precede macrovascular dysfunction, as it does in diabetics.25 Capillary rarefraction in the LV is a common finding in CKD.26 In advanced stages of CKD, microvascular structure or function may be obliterated by the time LV hypertrophy occurs, accounting for why VTI did not correlate with hypertrophy in patients with advanced disease. It is also notable that the FMD–LV mass association was attenuated by adjustment for sex. We are currently investigating associations of female or male sex with cardiovascular function in the cohort.

Association of Endothelial Dysfunction With Subclinical LV Diastolic Dysfunction

We show that in asymptomatic ESRD patients, both macrovascular (FMD) and microvascular function (VTI) are highly correlated with E/e’ ratio, a parameter that reflects LV diastolic function and preload. Given that patients were studied the morning after dialysis, that most patients were <0.5 kg over dry weight, and that hemoglobin on the study day was higher than on monthly laboratory tests, we conclude that participants were close to euvolemic. In this context, E/e’ ratio is less likely a maker of generalized fluid overload and more likely an indicator of intrinsic LV diastolic function. We also controlled for SBP in multivariate analyses. Thus, although we cannot determine the extent of confounding because of volume status, we conclude that the associations observed reflect correlations between endothelial and intrinsic LV diastolic function. These findings support current views28,29 on the importance of endothelial dysfunction and inflammation as likely synergistic precursors to diastolic dysfunction.24,29 CKD exemplifies a disease state of endothelial dysfunction30 and inflammation,31 and thus, CKD may be an especially appropriate population to study associations between endothelial dysfunction, inflammation, and abnormal systolic and diastolic cardiac mechanics. Further studies of inflammatory mediators in these patients might clarify the role of inflammation in the associations that we observed. Additional mediators known to correlate with endothelial dysfunction and left ventricular hypertrophy, including asymmetrical dimethylarginine32,33 and phosphorus34,35 could also play an important role; we plan further analyses of our data to examine associations between these factors and macro- and microvascular dysfunction. It is notable that, in contrast to the finding that both FMD and VTI were associated with LV diastolic dysfunction, neither was associated with systolic function as measured by global longitudinal strain or ejection fraction. Several possible explanations exist, including type II error due to modest sample size; however, we note that the effect sizes observed are not consistent with a clinically meaningful association, and thus we would not expect greatly different results with a larger sample size. This cohort sampled relatively healthy ESRD patients with mostly normal ejection fraction, which could have affected our ability to detect correlations with ejection fraction. Alternatively, our findings could be interpreted as showing that endothelial function is a contributor to diastolic dysfunction, but that other factors (for example, volume status or anemia) are more important determinants of systolic function in ESRD.

Association of Microvascular Function (VTI) With RV function

Animal studies have suggested that pulmonary microvascular remodeling may lead to increased RV afterload36 and influence RV function.37 Epidemiological studies in non-CKD populations have shown that RV dysfunction is particularly common in the setting of heart failure with preserved ejection fraction.38,39 Recent work by Shah et al40 has shown that RV remodeling is an independent prognostic factor in heart failure with preserved ejection fraction; in the same cohort, microalbuminuria (a marker of endothelial dysfunction) was associated with RV remodeling independent of comorbidities and LV mass.41 To our knowledge, the association of VTI with RV function that we observed has not been demonstrated previously in CKD or non-CKD populations. Notably, the association between VTI and RV dysfunction persists even in the smaller subgroup without self-reported atherosclerotic disease and in the whole group after adjustment for medications. These findings suggest that there is a particularly robust association between microvascular dysfunction and RV dysfunction. In light of current literature indicating that RV dysfunction precedes LV dysfunction and assuming that microvascular disease occurs before macrovascular disease, as in diabetes mellitus,25 it is possible that microvascular disease mediates RV dysfunction in early preclinical stages of heart failure.

Strengths

Our study has several strengths. The cohort is well characterized and is diverse with regard to age, sex, and race. We
captured a broad range of subclinical CVD by including participants actively waitlisted for kidney transplant, who by definition do not have severe CVD, as well as participants with more severe disease. There are several potential reasons why we found these associations between endothelial function and cardiac function, whereas a previous ESRD study did not. Study visits for hemodialysis patients were systematically planned for the day after the first dialysis day of the week dialysis week (Tuesday or Wednesday morning), a study design that may have minimized variations in volume status. FMD poses numerous technical difficulties in ESRD, and we were fortunate to have one specially trained technician perform all FMD and VTI studies. Additionally, we used upper arm cuff occlusion, an accepted site of cuff occlusion that typically

Table 4. Multivariable Associations of FMD and VTI With Left Ventricular Mass and Diastolic Function (E/e′)

<table>
<thead>
<tr>
<th>Endothelial Parameter</th>
<th>LV Mass, %Estimate (95% CI)*</th>
<th>P</th>
<th>Diastolic Function Assessed by E/e′, %Estimate (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMD (per doubling)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>−4.5 (−7.7 to −0.99)</td>
<td>0.01</td>
<td>−5.5 (−10.4 to −0.29)</td>
<td>0.04</td>
</tr>
<tr>
<td>Model 1†</td>
<td>−4.6 (−7.7 to −0.99)</td>
<td>0.01</td>
<td>−5.6 (−10.4 to −0.29)</td>
<td>0.06</td>
</tr>
<tr>
<td>Model 2</td>
<td>−4.1 (−7.7 to −0.49)</td>
<td>0.03</td>
<td>−2.7 (−7.7 to 3.0)</td>
<td>0.31</td>
</tr>
<tr>
<td>Model 3</td>
<td>−2.4 (−5.8 to 1.2)</td>
<td>0.19</td>
<td>−3.5 (−6.6 to 2.0)</td>
<td>0.20</td>
</tr>
<tr>
<td>Model 4</td>
<td>−2.0 (−5.8 to 1.2)</td>
<td>0.28</td>
<td>−2.0 (−6.6 to 3.0)</td>
<td>0.40</td>
</tr>
<tr>
<td>VTI (per doubling)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>0.30 (−4.9 to 6.2)</td>
<td>0.9</td>
<td>−13.1 (−20.5 to −5.8)</td>
<td>0.001</td>
</tr>
<tr>
<td>Model 1</td>
<td>0.40 (−4.9 to 6.2)</td>
<td>0.9</td>
<td>−12.2 (−20.0 to −4.9)</td>
<td>0.003</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.0 (−4.9 to 7.3)</td>
<td>0.6</td>
<td>−10.4 (−17.3 to −2.0)</td>
<td>0.01</td>
</tr>
<tr>
<td>Model 3</td>
<td>−1.9 (−6.7 to 4.1)</td>
<td>0.50</td>
<td>−10.4 (−18.0 to −2.0)</td>
<td>0.01</td>
</tr>
<tr>
<td>Model 4</td>
<td>−1.0 (−6.7 to 5.1)</td>
<td>0.7</td>
<td>−9.5 (−16.0 to −1.0)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

CI indicates confidence interval; FMD, flow-mediated dilation, LV, left ventricular; and velocity time integral.

*Outcomes analyzed are log-transformed LV mass and E/e’ ratio; β coefficients are back transformed so estimates represent percent change in outcome per doubling of FMD or VTI.

†Model 1: Age adjusted. Model 2: 1+systolic blood pressure. Model 3: 2+systolic blood pressure, cause of renal failure, body mass index, and tobacco use. Model 4: 3+use of angiotensin-converting enzyme inhibitor or angiotensin receptor blocker.

Table 5. Multivariable Adjusted Associations of FMD and VTI with Right Ventricular Function

<table>
<thead>
<tr>
<th>Endothelial Parameter</th>
<th>RV Free Wall Systolic Longitudinal Strain, %Estimate (95% CI)*</th>
<th>P</th>
<th>RV Early Diastolic Strain Rate, %Estimate (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMD (per doubling)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>0.30 (−2.0 to 3.0)</td>
<td>0.8</td>
<td>2.7 (−0.98 to 7.3)</td>
<td>0.18</td>
</tr>
<tr>
<td>Model 1†</td>
<td>−0.09 (−3.0 to 2.0)</td>
<td>0.9</td>
<td>2.0 (−0.98 to 6.2)</td>
<td>0.3</td>
</tr>
<tr>
<td>Model 2</td>
<td>−0.29 (−2.0 to 2.0)</td>
<td>0.8</td>
<td>2.0 (−1.9 to 5.1)</td>
<td>0.4</td>
</tr>
<tr>
<td>Model 3</td>
<td>−0.6 (−3.0 to 2.0)</td>
<td>0.6</td>
<td>0.30 (−3.9 to 5.1)</td>
<td>0.9</td>
</tr>
<tr>
<td>Model 4</td>
<td>−0.70 (−4.0 to 2.0)</td>
<td>0.6</td>
<td>−1.0 (−5.8 to 4.1)</td>
<td>0.6</td>
</tr>
<tr>
<td>VTI (per doubling)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>7.3 (3.0 to 11.6)</td>
<td>0.001</td>
<td>8.3 (1.0 to 16.1)</td>
<td>0.02</td>
</tr>
<tr>
<td>Model 1</td>
<td>6.2 (2.0 to 10.5)</td>
<td>0.004</td>
<td>6.2 (−0.7 to 13.8)</td>
<td>0.08</td>
</tr>
<tr>
<td>Model 2</td>
<td>5.1 (2.0 to 10.5)</td>
<td>0.007</td>
<td>5.1 (−1.8 to 13.9)</td>
<td>0.09</td>
</tr>
<tr>
<td>Model 3</td>
<td>5.1 (1.0 to 9.4)</td>
<td>0.02</td>
<td>8.2 (0.2 to 17)</td>
<td>0.04</td>
</tr>
<tr>
<td>Model 4</td>
<td>6.7 (2.0 to 11.6)</td>
<td>0.003</td>
<td>11.6 (3.0 to 21)</td>
<td>0.007</td>
</tr>
</tbody>
</table>

CI indicates confidence interval; FMD, flow-mediated dilation, RV, right ventricular; and velocity time integral.

*Outcomes analyzed are log-transformed LV mass and E/e’ ratio; β coefficients are back transformed so estimates represent percent change in outcome per doubling of FMD or VTI.

†Model 1: Age adjusted. Model 2: 1+systolic blood pressure. Model 3: 2+systolic blood pressure, cause of renal failure, body mass index, and tobacco use. Model 4: 3+use of angiotensin-converting enzyme inhibitor or angiotensin receptor blocker.
results in FMD values twice as large as the more distal placement; this method detected a broad range of endothelial function among our participants.

**Limitations**

In addition to these strengths, our study has several limitations. Indices of cardiac function, and in particular RV mechanics, are load dependent. Invasively measured central pressures are not available for these analyses, and thus, we cannot determine whether the observed associations are independent of volume status. However, we did adjust for peripheral and pulmonary SBP. Similarly, invasively measured coronary endothelial function was not measured; however, the correlation between peripheral FMD and coronary endothelial function in previous studies supports our analyses. Because we did not include a control group, our results are specific to ESRD, and further studies would be required to find out whether these associations hold true in populations with less advanced or no kidney disease. Participants are likely to represent the most medically compliant patients, judging by their euvolemic status and well-controlled blood pressure, and results may not generalize to patients with greater disease burden or higher rates of noncompliance. There were 11 participants with current tobacco use, and although we did adjust for smoking pack-years and asked patients to abstain from smoking for 12 hours before the study, the effects of smoking probably influenced these patients' endothelial dysfunction. Additional factors likely to affect endothelial function, which were not available for these analyses, include onset of hypertension and dosage of erythropoietin-stimulating agents. Our cross-sectional results show associations but cannot establish a causal relationship between endothelial function and cardiac function; longitudinal studies are needed to demonstrate whether endothelial function predicts decline in cardiac function in these patients.

**Perspectives**

Our data show that among asymptomatic, well-controlled, patients on dialysis, macrovascular endothelial dysfunction (FMD) is independently associated with LV mass. Microvascular endothelial dysfunction (VTI) is independently associated with LV diastolic dysfunction and RV systolic dysfunction. Studies of heart failure with preserved ejection fraction or patients with less advanced CKD might elucidate whether the VTI correlations with RV dysfunction and LV diastolic dysfunction apply to other populations. Our study provides a foundation for future interventional trials to determine whether novel endothelial therapies could slow the progression of LV hypertrophy or prevent heart failure in the ESRD population. Of particular interest are novel therapies that act downstream of nitric oxide synthase and could potentially obviate the issue of nitrate tolerance.

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**Disclosures**

None.

**References**

Novelty and Significance

What Is New?

To our knowledge, this is the first study in end-stage renal disease in which both macro- and microvascular endothelial function have been measured simultaneously with modern echocardiographic measures of systolic and diastolic cardiac function. We show that microvascular function is associated with diastolic left ventricular and systolic right ventricular dysfunction independently of comorbidities and hemodynamics.

What Is Relevant?

Diastolic and right ventricular dysfunction are increasingly recognized as integral to the development of heart failure with preserved ejection fraction, which is itself a common, highly morbid condition that is especially in chronic kidney disease and end-stage renal disease. Our results provide a foundation for trials of endothelial promoting therapeutics to prevent heart failure in chronic kidney disease, for which short-term surrogate outcomes might include endothelial function or speckle-tracking echocardiography.

Summary

In this ongoing, prospective study of patients with end-stage renal disease, baseline studies show strong correlations between microvascular function and both left ventricular and right ventricular dysfunction. Our results yield insight into the range and reproducibility of endothelial and cardiac function in this population and provide rationale for investigations of endothelial promoting therapies that could prevent heart failure in this population.
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ASSOCIATIONS OF MACRO- AND MICROVASCULAR ENDOTHELIAL DYSFUNCTION WITH SUBCLINICAL VENTRICULAR DYSFUNCTION IN END-STAGE RENAL DISEASE

Ruth F Dubin, MD¹; Isabella Guajardo¹; Amrita Ayer¹; Claire Mills²; Catherine Donovan²; Lauren Beussink³; Rebecca Scherzer, PhD¹; Peter Ganz, MD²; Sanjiv J Shah, MD³

1. San Francisco VA Medical Center/University of California, San Francisco
2. Center for Vascular Excellence, Division of Cardiology, San Francisco General Hospital/University of California, San Francisco
3. Division of Cardiology, Department of Medicine, Northwestern University Feinberg School of Medicine

Corresponding Author: Ruth Dubin, MD
San Francisco VA Medical Center
4150 Clement St. Box 111A1
San Francisco, CA  94121
Phone: (510) 847-4955
Fax: (415) 379-5573
Email: ruth.dubin@ucsf.edu
S1: Methods for Endothelium-dependent FMD and Echocardiography

Endothelial-dependent FMD was performed by ultrasound on the arm free of vascular access or other surgical intervention. Patients were instructed to take their regular medications, to avoid heavy meal, caffeine or smoking 12 hours prior to the FMD study (compliance with these instructions was 84%, 89%, 90% and 100%, respectively). Studies were performed in the morning, on either Tuesday or Wednesday (for hemodialysis patients, the day following the first dialysis session of the week). Blood pressure measurement was performed by a trained technician (CM) with a sphygmomanometer on the arm free of prior vascular surgery. Participants rested supine for 10 minutes in a quiet, temperature-controlled room, and then peripheral systolic blood pressure (SBP) was measured by automated sphygmomanometer using an appropriately sized cuff placed proximal antecubital fossa. Blood pressure was measured at two time points, 5 minutes apart. If the two blood pressures differed by more than 10 mmHg, the measurement was repeated every 5 minutes until repeat systolic blood pressures were within 10 mmHg, and the final blood pressure was noted as the blood pressure obtained prior to FMD. FMD was performed only on patients with an arm free of prior vascular access (functioning or not), by first occluding blood flow in the arm with a blood pressure cuff, inflated to at least 50 mmHg above peripheral SBP. Anticipating that FMD values would be very low in an ESRD cohort, we used upper arm cuff occlusion, an accepted site of cuff occlusion that typically results in FMD values twice as large as the more distal placement.\(^1\)

A single ultrasound technician (CM), who performed all FMD study procedures, confirmed whether the artery was fully compressible at 50 mmHg above SBP, using 2D imaging and Doppler, up to a maximum inflation pressure of 300 mmHg. If the artery was not fully compressible at 300 mmHg (as occurred in 7 participants), this was noted, and the study was performed using a cuff pressure of 300 mmHg; for most participants, the occlusion cuff pressure was between 240 mmHg and 300 mmHg. The ultrasound technician recorded baseline 2D and Doppler images of the brachial artery, and then the cuff was inflated for 5 minutes. Doppler flow was recorded immediately following cuff release; FMD was measured at 60 seconds and 90 seconds following cuff deflation.

FMD images were de-identified and transferred in DICOM format to the SFGH Center for Excellence in Vascular Research for analysis. Brachial artery diameters before and after cuff deflation were measured manually from the intima-media interfaces using MIA software (Medical Imaging Applications, Coralville, Iowa). The 60-second FMD image was used for all patients except for two, whose 90-second image was significantly clearer than at 60 seconds. Image quality was generally excellent; only 6 of 106 were not measured due to suboptimal imaging. FMD was measured as \(\frac{(\text{post-inflation diameter}) - (\text{pre-inflation diameter})}{(\text{pre-inflation diameter})} \times 100\%\) at five consecutive R waves, and the final FMD value was calculated as the average of five beats.\(^2\) VTI was measured by manually tracing the pulse wave in the MIA software, and then calculated using MIA algorithms. Final VTI values were calculated as the average VTI of the first three full beats following cuff deflation.\(^{2,3}\) In the presence of
arrhythmia (nine patients), if at least 3 consecutive R waves for FMD and at least 2 for VTI could not be ascertained, these images were dropped from the analysis (2 for FMD, 3 for VTI). Study Flow Chart and Image Selection are summarized in the online supplement, Figure S1.

**Echocardiography**

Comprehensive echocardiography, including 2D, M-mode, Doppler, tissue Doppler, and STE, was performed within 2 hours of FMD by a single experienced echocardiography technician (CD) using a Vivid 7 ultrasound machine and 3.5 MHz transducer (GE Healthcare, Waukesha, WI). De-identified images were transferred in RAW format to Northwestern University Echocardiography Core Laboratory and analyzed using GE EchoPac software version 7.0.0 (GE Healthcare, Waukesha, WI). Of the 152 echocardiograms, none were excluded due to poor image quality. Three echocardiograms were excluded due to greater than moderate valvular disease, leaving 149 echocardiograms for this analysis. Atrial fibrillation was present in one patient and ventricular ectopy in eight patients, but the presence of an abnormal cardiac rhythm did not prohibit quantitation of echocardiographic parameters. All measurements were performed by an experienced research sonographer (LB) and verified by an experienced investigator with expertise in echocardiography (SJS), according to guidelines of the American Society of Echocardiography.\(^{4,6}\) LV mass index was calculated using body surface area. Early (e’) diastolic tissue velocities were obtained in the apical 4-chamber view at the septal mitral annulus. Diastolic dysfunction was categorized according to the following criteria: normal=septal e’≥8cm/s; mild=septal e’<8cm/s, mitral deceleration time (dt)>200ms, E/A<0.8; moderate=e’<8cm/s, mitral dt 160-200ms, E/A 0.8-1.5; severe= e’<8cm/s, mitral dt≤160ms, E/A≥2.\(^{5}\) All strain parameters were reported as absolute values for ease of analysis and presentation; higher values represent better function.

**References**


Table S1: Characteristics of Patients Above and Below Median VTI or FMD

<table>
<thead>
<tr>
<th>Patient characteristic</th>
<th>VTI&lt;67cm (N=59)</th>
<th>VTI≥67cm (N=58)</th>
<th>p*</th>
<th>FMD&lt;4.3% (N=63)</th>
<th>FMD≥4.3% (N=60)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>59(12)</td>
<td>52(14)</td>
<td>0.02</td>
<td>57(12)</td>
<td>53(14)</td>
<td>0.17</td>
</tr>
<tr>
<td>Female gender</td>
<td>22(37%)</td>
<td>16(28%)</td>
<td>0.26</td>
<td>19(30%)</td>
<td>24(40%)</td>
<td>0.25</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>18(31%)</td>
<td>15(26%)</td>
<td>0.008</td>
<td>17(27%)</td>
<td>17(28%)</td>
<td>0.015</td>
</tr>
<tr>
<td>African American</td>
<td>25(42%)</td>
<td>12(21%)</td>
<td></td>
<td>28(44%)</td>
<td>13(22%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>16(27%)</td>
<td>31(53%)</td>
<td></td>
<td>18(29%)</td>
<td>30(50%)</td>
<td></td>
</tr>
<tr>
<td>Months on dialysis</td>
<td>55(44)</td>
<td>46(41)</td>
<td>0.27</td>
<td>50(43)</td>
<td>49(42)</td>
<td>0.95</td>
</tr>
<tr>
<td>History of tobacco</td>
<td>23(39%)</td>
<td>30(52%)</td>
<td>0.17</td>
<td>29(46%)</td>
<td>28(47%)</td>
<td>0.94</td>
</tr>
<tr>
<td>Diabetes</td>
<td>26(44%)</td>
<td>28(48%)</td>
<td>0.65</td>
<td>36(57%)</td>
<td>21(35%)</td>
<td>0.01</td>
</tr>
<tr>
<td>History of atherosclerotic disease†</td>
<td>23(39%)</td>
<td>11(19%)</td>
<td>0.017</td>
<td>22(35%)</td>
<td>15(25%)</td>
<td>0.23</td>
</tr>
<tr>
<td>History of heart failure</td>
<td>12(20%)</td>
<td>6(10%)</td>
<td>0.13</td>
<td>9(14%)</td>
<td>11(18%)</td>
<td>0.54</td>
</tr>
<tr>
<td>Peritoneal dialysis</td>
<td>4(7%)</td>
<td>10(17%)</td>
<td>0.08</td>
<td>5(8%)</td>
<td>9(15%)</td>
<td>0.22</td>
</tr>
<tr>
<td>Current dialysis access is graft</td>
<td>38(78%)</td>
<td>29(73%)</td>
<td>0.58</td>
<td>36(78%)</td>
<td>31(67%)</td>
<td>0.24</td>
</tr>
</tbody>
</table>
or fistula

**Cause of renal failure**

<table>
<thead>
<tr>
<th>Cause</th>
<th>17(29%)</th>
<th>23(40%)</th>
<th>0.73</th>
<th>30(48%)</th>
<th>12(20%)</th>
<th>0.003</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>16(28%)</td>
<td>11(19%)</td>
<td></td>
<td>17(27%)</td>
<td>13(22%)</td>
<td></td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>3(5%)</td>
<td>2(4%)</td>
<td></td>
<td>2(3%)</td>
<td>3(5%)</td>
<td></td>
</tr>
<tr>
<td>Other cause</td>
<td>8(14%)</td>
<td>8(14%)</td>
<td></td>
<td>3(5%)</td>
<td>13(22%)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>14(24%)</td>
<td>14(24%)</td>
<td></td>
<td>11(18%)</td>
<td>18(31%)</td>
<td></td>
</tr>
<tr>
<td>Active on transplant list</td>
<td>23(39%)</td>
<td>14(24%)</td>
<td>0.33</td>
<td>20(32%)</td>
<td>18(30%)</td>
<td>0.3</td>
</tr>
<tr>
<td>Anuric</td>
<td>16(28%)</td>
<td>14(24%)</td>
<td>0.67</td>
<td>13(21%)</td>
<td>17(28%)</td>
<td>0.3</td>
</tr>
<tr>
<td>SBP prior to fmd (mmHg)</td>
<td>134(21)</td>
<td>132(22)</td>
<td>0.53</td>
<td>139(21)</td>
<td>126(20)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DBP prior to fmd (mmHg)</td>
<td>77(22)</td>
<td>76(12)</td>
<td>0.96</td>
<td>77(21)</td>
<td>76(13)</td>
<td>0.52</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>21(5.7)</td>
<td>22(7.4)</td>
<td>0.61</td>
<td>22(6.0)</td>
<td>21(7.2)</td>
<td>0.020</td>
</tr>
<tr>
<td>Most recent post-dialysis</td>
<td>0.49(1.2)</td>
<td>0.34(1.2)</td>
<td>0.97</td>
<td>0.26(1.6)</td>
<td>0.49(0.68)</td>
<td>0.11</td>
</tr>
<tr>
<td>weight – dry weight (kg)†</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

*P*-values obtained with chi2 for categorical and with wilcoxon rank-sum for continuous variables. † atherosclerosis defined as self-report of myocardial infarction, stroke, percutaneous coronary intervention, coronary artery bypass graft, or
peripheral artery disease. ‡ Represents the degree of volume overload for hemodialysis patients at the last dialysis session, which was the day prior to study visit
Figure S1: Study Flow Chart and Image Selection

Total participants who underwent echocardiography (N=152)

Excluded from analysis:
- More than moderate valvular disease (N=3)
- Bilateral vascular access precluding FMD measurement (N=20)

Total participants who underwent echocardiography and endothelial testing (N=129)

FMD or VTI images were excluded for three main reasons (overlapping categories):
- Unclear FMD image (N=3)
- Arrhythmia precluding measurement of FMD (N=3)
- Inadequate number of beats for VTI (N=8)

Total participants with measurable echocardiogram, FMD (N=123) and VTI (N=117)