Impact of Frequent Nocturnal Hemodialysis on Myocardial Mechanics and Cardiomyocyte Gene Expression

Christopher T. Chan, MD; Sara Arab, PhD; Shemy Carasso, MD; Gil Moravsky, MD; Guo Hua Li, PhD; Peter P. Liu, MD*; Harry Rakowski, MD*

Background—Regression of left ventricular mass with nocturnal hemodialysis has been observed. The influence of nocturnal hemodialysis on myocardial mechanics and cardiomyocyte gene expression is unknown.

Methods and Results—Forty-two patients (30 male:12 female; age, 44±12 years [mean±SD]) with end-stage renal disease were followed for 3.1±1.8 years before and after conversion to nocturnal hemodialysis and were compared with 29 normal subjects (18 male:11 female; age, 48±13 years). Myocardial mechanics were assessed by 2-dimensional velocity vector imaging. Uremic plasma (10%) was added to cultures of neonatal Sprague-Dawley rat ventricular myocytes. Total RNA was isolated from cell cultures and subjected to differential gene expression profiling with specific interest in genes affecting apoptosis and fibrosis. Left ventricular mass index and left atrial volume index decreased from 122.6±42.6 to 98.5±34.9 g/m² (P<0.001) and 25.9±9.1 to 22.5±9.6 cm³/m² (P=0.005), respectively. Left ventricular apical circumferential strain and basal rotation improved after conversion to nocturnal hemodialysis and approximated normal values. Nocturnal hemodialysis increased sessional dialysis dose and lowered parathyroid hormone levels (from 51±67 to 24±37 pmol/L, P<0.05) and phosphate. Under conventional hemodialysis conditions, there was an upregulation of genes leading to apoptosis and fibrosis in cardiomyocytes. The change in left ventricle rotation was associated with the change in parathyroid hormone values (r=0.37, P=0.02) and to the change in left ventricle mass (r=0.31, P=0.046).

Conclusions—Frequent hemodialysis is associated with improvement in myocardial mechanics and cardiac gene expression profile, which warrants prognostic validation. (Circ Cardiovasc Imaging. 2012;5:474-480.)

Key Words: cardiomyocyte gene expression | diastolic function | frequent hemodialysis | myocardial mechanics

Left ventricular hypertrophy is prevalent in end-stage renal disease (ESRD) and contributes to the high annual mortality rate seen in patients on dialysis. Although conventional hemodialysis (CHD; 3 times per week, 3–4 hours per session) is the standard renal replacement therapy in North America, it does not correct abnormal left ventricular geometry.

Clinical Perspective on p 480

Epidemiological and experimental studies have documented the rising burden of diastolic heart failure as a consequence of impaired left ventricular (LV) compliance, which has been in part attributed to hypertrophy and fibrosis of the left ventricle. Multiple risk factors are known to be associated with left ventricular hypertrophy in ESRD including volume and pressure overload, vascular stiffening, and neurohormonal activation. Similarly, several mediators of myocardial fibrosis have been implicated in ESRD, namely, increased myocardial collagen turnover, cardiac ischemia, norepinephrine, angiotensin II, aldosterone, and parathyroid hormone (PTH). Of interest, PTH is known to activate the interstitial cell leading to cardiac fibrosis. Most recently, other mediators of calcium/phosphate metabolism such as fibroblast growth factor 23 were also shown to correlate with left ventricular hypertrophy in patients with chronic kidney disease and were shown to be an independent predictor of death in the dialysis population. Hence, genes that lead to cardiomyocyte fibrosis and apoptosis are of particular mechanistic interest.

Two-dimensional deformation analysis is a tool to quantify regional and global ventricular mechanics by echocardiography. LV strain and rotation derived from 2-dimensional deformation analysis have been applied in multiple cardiac populations and were shown to have significant prognostic value in high-risk individuals such as heart transplant recipients.

Given that observational and randomized controlled studies have documented regression of left ventricular mass by nocturnal home hemodialysis (NHD; 5–6 sessions per week,
6–8 hours per session), we hypothesize that NHD (compared with CHD) may improve myocardial function as assessed by myocardial mechanics. In addition, we hypothesize that soluble factors within the serum obtained from patients with ESRD before and after conversion from CHD to NHD are responsible for changes in cardiomyocyte gene expression patterns (with specific interest in genes leading to fibrosis and apoptosis), which are associated with an improvement in left ventricular geometry in ESRD before and after conversion from CHD to NHD.

Methods
This protocol was approved by the Research Ethics Board of the Toronto General Hospital, University Health Network, Toronto, Canada, and conformed to the standards established by the Declaration of Helsinki. Medically stable patients with ESRD (age between 18 and 85 years) who had received a minimum of 3 months of CHD and were training for NHD were invited to participate in this study. None of the patients had any acute illness, hospitalization, or symptomatic cardiovascular disease (including congestive heart failure and acute coronary syndrome). Normal subjects were recruited for echo-cardiography to evaluate minor symptoms or murmurs who had normal echocardiographic findings and healthy volunteers. Twenty-nine subjects matched for age and sex distribution (18 male:11 female; age, 48±13 years) were included in the present study (Table 1). Normal subjects were derived from healthy subjects referred for echocardiography to evaluate minor symptoms or murmurs who had normal echocardiographic findings and healthy volunteers. Twenty-nine subjects matched for age and sex distribution (18 male:11 female; age, 48±13 years) were included in the present study (Table 1).

Dialysis Protocol
Patients on NHD received hemodialysis at home for 6 to 8 hours 5 to 6 nights per week. Vascular access was achieved through either a long-term internal jugular catheter (Uldall Catheter; Cook Critical Care, Bloomington, IN) or an arteriovenous fistula. Dialysate flow rate of 350 mL/min and blood flow rate of 200 to 300 mL/min were used. F80 polysulfone dialyzers (Fresenius Medical Care, Lexington, MA) or Excella 120 dialyzers (Baxter, Chicago, IL) were used. Patients on CHD received hemodialysis for 4 hours 3 times per week through similar vascular access. A blood flow rate of 400 mL/min, a dialysate flow rate of 500 to 750 mL/min, and F80 polysulfone dialyzers (Fresenius Medical Care) were used. Unfractionated heparin was used for anticoagulation on CHD and NHD.

Dialysis dose per treatment was estimated by equilibrated Kt/V (eKt/V) as described by Daugirdas and colleagues in which eKt/V = (spKt/V−0,6×(spKt/V))/t+0,03 (spKt/V=single pool Kt/V; K=delivered clearance; t=dialysis time; and V=urea distribution volume). Single pool Kt/V was determined using blood urea reduction ratio.23

Echocardiographic Doppler Studies
Echocardiographic studies were interpreted by a cardiologist blinded to patients’ dialysis prescription. Two-dimensional, Doppler, and tissue Doppler imaging parameters were measured according to the guidelines of the American Society of Echocardiography.24 Left atrial (LA) pressure was estimated using the Nagueh method.25

Strain, Strain Rate, and Rotation Evaluation
Measurements were performed using velocity vector imaging software (Siemens Medical Systems, Mountainview, CA).16,18,24 Velocity
vector imaging quantified myocardial motion from B-mode clips by automatically tracking user-defined points to define the inward and outward motion of the myocardial subendocardial regions. Two-dimensional tissue velocity was computed. Strain and strain rate were computed by the change in the relative distance between localized tracked trace points. Strain was defined as the instantaneous local trace lengthening or shortening and strain rate as the rate of lengthening-shortening. Circumferential strain, strain rate, and rotation were measured at 3 parasternal short axis planes (base, mid, and apex, 6 segments per level). Longitudinal wall strains and strain rates were measured from the apical 2-, 3-, and 4-chamber views, 6 segments per view. As a measure of diastolic relaxation, we measured the early apical reverse rotation angle difference—from peak rotation to 10% into diastole.

Intraobserver Variability

Two-dimensional strain measurements were done by one observer (S.C.). We reanalyzed 10 studies to evaluate intraobserver variability. For peak strain, the intraobserver agreement was 0.9.

Gene Expression Protocol

Neonatal Rat Ventricular Myocyte Culture Preparation

Neonatal Sprague-Dawley (Charles River, Montreal, Canada) rat ventricular myocytes were isolated and cultured as described previously. A single litter of 1- to 2-day-old rats was used for each experiment. Pups were sacrificed by cervical dislocation and the hearts removed quickly into filter-sterilized buffer. Using an aseptic technique, atria and blood vessels were removed and the ventricles minced. Ventricular tissue was dissociated at room temperature previously. A single litter of 1- to 2-day-old rats was used for each experiment. We reanalyzed 10 studies to evaluate intraobserver variability. For peak strain, the intraobserver agreement was 0.9.

Gene Expression Profiling

Total RNA was isolated from cells (46 samples) using Trizol Reagent (GIBCO/BRL) following the manufacturer’s protocol on Day 5. The quality of total RNA was assessed by an Agilent 2100 Bioanalyzer (Version A.02.0151232; Agilent Technologies). Only RNA with the outer diameter ratio of 1.99 to 2.0 at 260/280 was used for microarray analysis.

Results

Forty-two patients (30 male:12 female; age, 44±12 years) with ESRD were studied. The mean time of follow-up was 3.1±1.8 years. Their comorbid conditions were listed in Table 1. Ninety percent of our study population had a diagnosis of hypertension and was prescribed antihypertensive medications. Twenty-nine subjects matched for age and sex distribution (18 male:11 female; age, 48±13 years) were studied. None of the normal subjects had any chronic illness.

NHD increased sesselional dialysis dose and frequency doubled. In addition, NHD lowered parathyroid hormone levels (from 51±67 to 24±37 pmol/L, P<0.05) and phosphate concentration (from 1.42±0.4 to 1.24±0.4 mmol/L, P<0.05). Despite having a significant reduction in the number of antihypertensive medications (2.5–0.5 classes per patient, P<0.05), systolic blood pressure tended to fall (from 132±20 to 124±14, P=0.07) and diastolic blood pressure fell (from 81±11 to 75±10, P=0.01) after conversion to NHD (Table 2). Differences in systolic and diastolic mechanics were presented in Table 3. At baseline, compared with normal subjects, patients with ESRD exhibited greater LV mass, LA diameter, and elevated mitral E and A velocities. After conversion to NHD, LV mass index and LA volume index decreased from 122.6±42.6 to 98.5±34.9 g/m² (P<0.001) and 25.9±9.1 to 22.5±9.6 cm²/m² (P=0.005), respectively. Mitral E and A velocities tended to decrease (P=0.1 and P=0.08, respectively). Average longitudinal strain did not change between treatment groups and remained mildly decreased function, biological process, and cellular component. Raw data from microarray experiments was submitted to the Gene Expression Omnibus database (www.ncbi.nlm.nih.gov/geo) and the GEO accession ID is GSE28723.
Table 3. Echocardiographic Variables in Normal Controls and Patients With End-Stage Renal Disease Before and After Conversion To Nocturnal Hemodialysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal (n=29)</th>
<th>CHD (n=42)</th>
<th>NHD (n=42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV mass, g</td>
<td>130±25</td>
<td>224±84†</td>
<td>184±70‡</td>
</tr>
<tr>
<td>LVMI, g/m²</td>
<td>71±11</td>
<td>121±43†</td>
<td>98±35‡</td>
</tr>
<tr>
<td>LA systolic diameter, cm</td>
<td>3.5±0.3</td>
<td>4.0±4†</td>
<td>3.7±6*</td>
</tr>
<tr>
<td>Mitral E wave velocity, m/s</td>
<td>0.73±0.2</td>
<td>0.88±0.3†</td>
<td>0.80±0.2</td>
</tr>
<tr>
<td>Mitral A wave velocity, m/s</td>
<td>0.68±0.2</td>
<td>0.82±0.3†</td>
<td>0.74±0.2</td>
</tr>
<tr>
<td>Mitral annular E’ velocity, m/s</td>
<td>0.10±0.02</td>
<td>0.13±0.05†</td>
<td>0.14±0.05</td>
</tr>
<tr>
<td>LV longitudinal strain, %</td>
<td>−20±2</td>
<td>−17±4†</td>
<td>−17±4‡</td>
</tr>
<tr>
<td>LV base circumferential strain, %</td>
<td>−25±4</td>
<td>−21±6*</td>
<td>−21±6‡</td>
</tr>
<tr>
<td>LV apex circumferential strain, %</td>
<td>−29±4</td>
<td>−26±8†</td>
<td>−29±7*</td>
</tr>
<tr>
<td>Base rotation, °</td>
<td>−4.0±2.5</td>
<td>−5.9±2.6†</td>
<td>−4.7±2.6*</td>
</tr>
<tr>
<td>Apex rotation, °</td>
<td>7.2±3.6</td>
<td>5.4±3.0†</td>
<td>6.8±3.3*</td>
</tr>
<tr>
<td>Apical early diastolic (10%) reverse rotation, °</td>
<td>4.33±2.14</td>
<td>4.08±2.55</td>
<td>5.38±2.68*</td>
</tr>
</tbody>
</table>

Data represented as mean±SD.

*P<0.05 between CHD and normal.
†P<0.05 between CHD and NHD.
‡P<0.05 between normal and NHD.

The importance of impaired LV compliance in the ESRD population has led to extensive search for accurate noninvasive methods of quantifying its severity. To date, there is no single validated gold standard to assess for impaired LV relaxation. Traditionally, transmitral Doppler E/A ratio has been used to quantify the degree of impairment in LV relaxation. However, there is a wealth of published literature that has suggested that there is significant dependence between cardiac preload conditions and E/A ratio, especially in patients with ESRD.31,32 Given that there is a complex interaction between cardiac preload and “dry weight” in all patients with ESRD, E/A ratio may be a suboptimal measure to quantify the severity of LV noncompliance. The examination of LV mechanics may quantify the severity of impaired LV relaxation without the potential confounding effect of extracellular volume control. Furthermore, several investigators have proposed that analysis of LV myocardial mechanics may provide a more sensitive examination of LV compliance before a change in E/A ratio occurred.33,34 In the present report, we observed improvements in several indices of LV mechanics, regression of LV mass, and reduction of LA volume index. Myocardial shortening occurs in both the longitudinal and circumferential planes with a normal base to apex strain gradient with higher apical circumferential strain. Abnormalities in myocardial mechanics have been shown to occur well before changes in LV ejection fraction. In this study, with more frequent dialysis, longitudinal strain remained mildly decreased but apical circumferential strain increased in keeping with improving systolic myocardial function. Basal rotation returned to normal levels with increased apical rotation, thus improving the wringing action of the myocardi um in systole. It is reasonable to speculate that left ventricular circumferential function (strain, rotation) is the principal compensatory mechanism that preserves LV systolic function as reported in other cardiac pathologies.19,33,34 Improvement in diastolic myocardial performance also occurred with faster reverse rotation in early diastole, a finding that is relatively independent of filling pressures. Structurally, myocardial fiber orientation spans vertically at the subendocardium and horizontally at the subepicardium to maintain longitudinal and circumferential LV function, respectively. Thus, localized abnormalities may interfere with longitudinal and circumferential function differentially.35–38

Our results suggest that there is a potential mechanistic link among PTH control, LV mechanics, and LV mass. Hyperphosphatemia and hyperparathyroidism are established independent cardiovascular risk factors in ESRD.39 It is generally accepted that hyperphosphatemia is an important factor in the development of uremic calcific vasculopathy, which in turn will increase cardiac afterload and contributes directly to the progression of left ventricular hypertrophy.40,41 Our present results support the notion that improved control of PTH may lead to a reduction in intermyocardial fibrosis, thereby resulting in improved LV myocardial mechanics.

Our genomic results also support the hypothesis that there are soluble factors in uremic serum, which may modify cardiac gene expression patterns. It is interesting to note that uremic serum was linked to genes responsible for apoptosis and fibrosis of cardiomyocytes, which in turn may result in impaired...
LV relaxation. In fact, expressions of proapoptotic factors (eg, Bax, p53, Fas) have been linked consistently with progression of heart failure in the non-ESRD population. In addition, our results suggest that contractility may be modified by NHD through upregulation of S100A1, which is a calcium binding protein that is expressed in cardiac muscle. S100A1 modulates calcium homeostasis, energy metabolism, and contractile performance of the heart. Downregulation of S100A1 in cardiomyocytes after myocardial infarction has been linked to reduced cardiac reserve and development of heart failure. It is equally intriguing to note that in a pilot cohort study, patients with ESRD and refractory heart failure exhibited improvement in ejection fraction after conversion from CHD to NHD.

In summary, we have evidence to support the concept that augmented uremia clearance using NHD is accompanied by improvement in LV strain, LV rotation, a reduction in LV mass, and LA volume index. Increases in frequency and duration of dialysis are also associated with a downregulation of genes responsible for cardiomyocyte apoptosis and fibrosis and an upregulation of S100A1, which may improve LV contractility. Our results are limited by their observational nature. Additional experiments using other cardiomyocyte functional assays and serum-mixing strategies are required to improve our basic understanding of the influence of uremia on cardiac function and geometry. Future work determining the influence of frequent hemodialysis on LV mass regression and the effects on cardiac gene expression is warranted. The use of a stable ESRD population and the lack of a randomized controlled design is consistent with the pilot nature of the results;

Table 4. Microarray Exploration of Genes That Have Altered Expression in Cardiomyocytes Under Different Uremic Conditions

<table>
<thead>
<tr>
<th>Gene</th>
<th>No. of Fold Change (CHD versus NHD)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cdkn1a (cyclin-dependent kinase inhibitor 1A)</td>
<td>2.1-fold increase (CHD)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cdkn1c (cyclin-dependent kinase inhibitor 1C)</td>
<td>2.7-fold increase (CHD)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fas</td>
<td>2.4-fold increase (CHD)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Bax (Bc12-associated X protein)</td>
<td>2.5-fold increase (CHD)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>S100a1 (S 100 calcium binding protein 1A)</td>
<td>2.1-fold increase (NHD)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

CHD indicates conventional hemodialysis; NHD, nocturnal hemodialysis.
however, given the important potential clinical implications of LV geometry, function, and genomic signature in ESRD, we believe our work adds support to the growing benefits of nocturnal hemodialysis.

Figure 2. Real-time quantitative polymerase chain reaction confirmation of cardiomyocyte gene signature before and after conversion from conventional to nocturnal hemodialysis. Black bar indicates conventional hemodialysis. White bar indicates nocturnal hemodialysis. **P<0.05 between conventional hemodialysis and nocturnal hemodialysis. Cdkn1a indicates cyclin-dependent kinase inhibitor 1A; Cdkn1c, cyclin-dependent kinase inhibitor 1C; Bax, Bcl2-associated X protein; S100a1, S 100 calcium binding protein A1.

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Disclosures
None.

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26. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise JS, Solomon SD, Spencer KT, Sutton MS, Stewart WJ. Recommendations for chamber quantification: a report from the American Society of Echocardiography’s Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. *J Am Soc Echocardiogr.* 2005;18:1440–1463.

**CLINICAL PERSPECTIVE**

Diastolic dysfunction is common in patients with end-stage renal disease. Myocardial mechanics were assessed by 2-dimensional velocity vector imaging in 29 normal subjects and in 42 patients with end-stage renal disease before and after conversion from conventional hemodialysis (3 times a week, 4 hours per session) to nocturnal hemodialysis (5–6 nights a week, 6–8 hours per session). Gene expression profile from neonatal Sprague-Dawley rat ventricular myocytes was also examined before and after conversion to nocturnal hemodialysis. With nocturnal hemodialysis, left ventricular mass index and left atrial volume index decreased. Left ventricular apical circumferential strain and basal rotation improved after conversion to nocturnal hemodialysis and approximated normal values. Under conventional hemodialysis conditions, there was an upregulation of genes leading to apoptosis and fibrosis in cardiomyocytes. More frequent hemodialysis could be expected to improve left ventricular mechanics in patients with end-stage renal disease, which may further prevent important cardiovascular complications.
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**Supplemental Table.** Primers used for Real Time Quantitative PCR

<table>
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<tr>
<th>Gene</th>
<th>PF</th>
<th>PR</th>
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<tbody>
<tr>
<td>Cdkn1a</td>
<td>5’ – GCAGACCAGCCTGACAGATTT – 3’</td>
<td>5’ – CTCCAGACCCACACAGAAGA – 3’</td>
</tr>
<tr>
<td>Cdkn1c</td>
<td>5’ – AATCAGCCAGCCTCGACCAT – 3’</td>
<td>5’ – TGGGAAGGTATCGCTGGAGG – 3’</td>
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<tr>
<td>Fas</td>
<td>5’ - CGGTGGTATTTTTTATGGTTCTG - 3'</td>
<td>5’ - TGAACTCACGGAGTTCTGCCA - 3’</td>
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<tr>
<td>Bax</td>
<td>5’ - TGGAGCTGCAGAGGATGGATTG - 3'</td>
<td>5’ - CCCAGTTGAAGTTGCCATCAG - 3’</td>
</tr>
<tr>
<td>S100a1</td>
<td>5’- CCAACCGTGCTGCTGCTGAA –3’</td>
<td>5’ - TTTGTCCCTTCTTGCCC - 3’</td>
</tr>
</tbody>
</table>

Cdkn1a: cyclin-dependent kinase inhibitor 1A, Cdkn1c: cyclin-dependent kinase inhibitor 1C, Bax: Bcl2-associated X protein, S100a1: S 100 calcium binding protein A1