Chapter 9

# Reduced Renal Perfusion in Patients with Chronic Heart Failure is the Major Determinant of Renal Dysfunction and Related to Structural Renal Damage

Tom D.J. Smilde, Pim van der Harst, Dirk J. van Veldhuisen,

Gerjan Navis, Adriaan A. Voors, Frans Boomsma,

Daan B. Westenbrink and Hans L. Hillege

## ABSTRACT

*Introduction* Renal dysfunction in patients with chronic heart failure (CHF) is associated with increased morbidity and mortality. We studied determinants of renal dysfunction in patients with established CHF.

*Methods* We studied 86 patients with CHF, aged 58±12 years, LVEF 0.27±0.09. Glomerular filtration rate (GFR) was determined by iothalamate clearance. Urinary albumin excretion (UAE) was measured in 2x24 hours urine collections. We determined endothelial function (soluble vascular cell adhesion molecule 1 (sVCAM-1), soluble E-selectin (sES), Von Willebrand factor (vWF), nitrite/nitrate (NOx), asymmetric di-methyl arganine (ADMA)), inflammatory status (high sensitive C reactive protein (CRP)), renin angiotensin system parameters (plasma renin activity (PRA) and angiotensin II (AngII)), and hemodynamic status (N terminal pro brain natriuretic peptide (NT-pro BNP), mean arterial pressure (MAP), renal blood flow (RBF; Hippuran clearance/1-Hct).

*Results* Impaired endothelial function (sVCAM-1, vWF, NOx, ADMA), activation of the renin angiotensin system (PRA, Ang II), and impaired hemodynamics (NT pro-BNP, MAP, RBF) were all associated with a decreased GFR in heart failure (all $P < 0.05$), whereas systemic increased inflammatory status (CRP) was not. RBF was the critical determinant of GFR, accounting for 85% of variance in GFR. In patients with compromised renal function, UAE was increased exponentially.

*Conclusions* The present study shows that endothelial dysfunction and activation of the RAS are associated with renal dysfunction in CHF patients. The most important determinant of renal dysfunction is reduced renal perfusion, that was related to structural renal damage with elevated UAE.

## INTRODUCTION

Impaired renal function has consistently been found to be an independent risk marker for cardiovascular disease (CVD) outcomes and all-cause mortality in patients with chronic heart failure (CHF).[1;2] Several explanations have been provided for the strong prognostic value of renal function in CHF. First, impaired renal function can be considered to reflect reduced renal perfusion, as a consequence of impaired hemodynamic status, which is related to the severity of the underlying (i.e. cardiac) disease.[3;4] Even early in the course of CHF, when cardiac output is only reduced by 20%, renal perfusion can be substantially decreased up to 50%. This finding reflects intense renal vasoconstriction even in the initial stages of CHF. The specific factors that mediate renal vasoconstriction are not completely understood. Second, renal dysfunction might be a marker of general vascular disease and might reflect the severity of atherosclerotic disease.[5;6] Finally, impaired renal function might share common pathophysiological pathways with heart failure, such as endothelial dysfunction and increased inflammatory activity.[7;8] In CVD both endothelial function and inflammatory status are considered to be important risk factors for mortality. Accurate understanding of the link between CHF and renal dysfunction requires a systematic, comprehensive, assessment of the possible factors involved. Therefore, we assessed several of these factors in patients with a wide range of left ventricular dysfunction, and determined their relationship with impaired renal function. All patients used renine angiotensin system (RAS) inhibitors, which are essential in treatment of CHF. Our primary focus was the cardio-renal hemodynamics, biochemical markers of RAS-activity, endothelial dysfunction and inflammation. We also determined urinary albumin excretion (UAE). The presence of albuminuria is not only associated with an increased hemodynamic load and glomerular hyperfiltration but also with endothelial dysfunction. It predicts nephropathy and increased risk of cardiovascular morbidity and mortality in diabetic as well as in non-diabetic patients.[9-11]

## METHODS

*Patient population*

Outpatient CHF patients, aged ≥ 18 years, left ventricular ejection fraction (LVEF) < 45%, and clinically stable, were asked to participate. Patients had to use renin-angiotensin-system inhibitors, and all medication had to be stable for at least 1 month. Exclusion criteria included stroke or myocardial infarction within the last 3 months, cardiac surgery or angioplasty within the last 3 months or scheduled to undergo these procedures, unstable angina pectoris, primary renal disease, patients with prior organ transplant, or chronic use of medication compromising renal function. Care was taken to include patients over the full range of severity of CHF. In this study 102 patients were included. Sixteen patients were excluded for this analysis, because of missing hematocrit or UAE data. In total 86 patients were eligible for the current analysis.

*Study design*
All patients underwent the same study protocol. On the first day, glomerular filtration rate (GFR) and renal blood flow (RBF) were measured by the clearances of iothalamate and Hippuran as described below.[12] Body weight and length were determined just before renal function measurement started. In addition, during renal measurements, blood pressure and heart rate were determined. Systolic and diastolic blood pressure measurements were calculated as the mean of the last two out of ten consecutive measurements during ten minutes in sitting position with an automatic Dinamap XL Model 9300 series device (Johnson-Johnson Medical INC, Tampa, Florida). After the renal function measurements by iothalamate were finished, patients were given two containers for two times 24h urine collection. After return of these containers, samples were taken to determine urinary albumin concentration for calculation of 24h UAE.

*Renal function measurement by iothalamate clearance*
GFR and effective renal plasma flow (ERPF) were measured by constant infusion of radiolabelled tracers, $^{125}$I-iothalamate and $^{131}$I-Hippuran.[12] After drawing a blank blood sample, a priming solution containing 0.4 ml/kg body weight of the infusion solution (0.04 MBq of $^{125}$I-iothalamate and 0.03 MBq of $^{131}$I-hippuran) plus an extra of 0.6 MBq of $^{125}$I-iothalamate was given at 8 am, followed by infusion at 12 ml/h. In order to attain stable plasma levels of both tracers, a two hour stabilisation period followed, after which baseline measurements were started at 10 am. The clearances were calculated as (U•V)/P and (I•V)/P, respectively. U•V represented the urinary excretion of the tracer, I•V the infusion rate of the tracer; P the tracer value in plasma at the end of each clearance period. This method corrects for incomplete bladder emptying and dead space, by multiplying the urinary clearance of $^{125}$I-iothalamate with the ratio of the plasma and urinary clearance of $^{131}$I-Hippuran. This method has a day-to-day variation coefficient of 2.5% for GFR and 5% for ERPF. GFR and ERPF were corrected for 1.73m$^2$ of body surface area. Renal blood flow was calculated as ERPF / 1-hematocrit. The filtration fraction (FF) was calculated as the ratio of GFR and ERPF and expressed as percentage.

*Cardio-renal hemodynamic parameters*
RBF, FF, LVEF, Mean arterial pressure (MAP), and N-terminal pro_brain natriuretic peptide (NT pro_BNP) were used as markers for the cardio-renal hemodynamic status of the patients. MAP was calculated as follows; $^1/_3$ • systolic + $^2/_3$ • diastolic.

Urinary albumin concentrations were determined by nephelometry (Dade Behring Diagnostics, Marburg, Germany). Serum and urine creatinine was determined by Kodak Ektachem dry chemistry (Eastman Kodak, Rochester, NY, U.S.A.). NT pro_BNP was measured by electrochemiluminescence immunoassay on the Roche Elecsys (Roche diagnostics, Netherlands). Urinary albumin excretion was determined as the mean of two 24h urine collections.

We also determined the creatinine clearance and estimated GFR to evaluate whether the course of creatinine clearance and estimated GFR would be similar as the course of true GFR. Creatinine clearance (CrCl) was calculated as the mean of two 24-hour urine creatinine

excretions divided by plasma creatinine and corrected for 1.73m$^2$ of body surface area. The renal function was estimated using the simplified modification diet in renal disease formula[13] (eGFR= mL/min/1.73m$^2$;(186.3 • (serum creatinine)$^{-1.154}$ • (age)$^{-0.203}$, and • 0.742 if female). The body surface area (BSA) was calculated as follows: 0.007184 • weight$^{0.425}$ • length$^{0.725}$.

*Renin angiotensin system parameters*
Plasma renin activity (PRA) and angiotensin II (Ang II) were used as markers for RAS activity. PRA was measured by an immunoradiometric assay (Nichols Institute Diagnostics, Middlesex, United Kingdom). Ang II was measured by specific radioimmunoassays after SepPak extraction of plasma. Analyses were performed in a routine setting according to the guidelines of the manufacturer.

*Endothelial function parameters*
Von Willebrand factor (vWf), plasma nitrite/nitrate (NOx) and asymmetric di-methyl arganine (ADMA), soluble vascular adhesion molecule 1 (sVCAM-1), and soluble E-selectin (sES) were used as markers for endothelial damage and activation. vWF was determined using a validated in-house ELISA, as described previously.[14] NOx was determined in plasma after ultrafiltration through a 10 kDa molecular weight cut-off filter (Millipore BV). A colorimetric assay was used according to the instructions of the manufacturer (Cayman Chemical Company, Ann Abor, MI). ADMA was determined in plasma using a commercially available ELISA kit (DLD diagnostika GmbH, Hamburg, Germany) according to the instructions as supplied by the manufacturer. Serum levels of sVCAM-1 and sES were determined by commercially available ELISA kits (R&D Systems, Abingdon, UK and Bender Med Systems, Vienna, Austria, respectively) according to the manufacturer's instructions.

*Inflammation*
High sensitive C-reactive protein (CRP) was determined by nephelometry with a threshold of 0.156 mg/L and intra- and inter-assay coefficients of less than 4.4% and 5.7%, respectively (BNII N, Dade Behring, Marburg, Germany). CRP levels below the detection level were scored as 0.156 mg/L.

*Statistical analyses*
Correlation between GFR and various variables were performed using Pearson's correlation coefficients. Linear regression was performed to adjust for age and sex. Non-normally distributed continuous variables were log-transformed. We investigated the possibility of a curvilinear effect relation between RBF and GFR, FF, UAE, eGFR and CrCl by fitting of different fractional polynomial models. A $P < 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS, Chicago version 11 and STATA Statistical Software: release 8.2.

Table 1. Baseline characteristics for total study population

| | Total Population *(n=86)* |
|---|---|
| Age (years) | 58 ± 12 |
| NYHA class | 2.3 ± 0.8 |
| Sex (n, % male) | 67 (78) |
| Diabetes (n, %) | 7 (8) |
| Current smoking (n, %) | 14 (18) |
| Ischemic etiology (n, %) | 43 (50) |
| *Cardiorenal hemodynamic parameters* | |
| GFR (mL/min/1.73m$^2$) | 74 ± 28 |
| RBF (mL/min/1.73m$^2$) | 465 ± 161 |
| FF (%) | 27 ± 5 |
| LVEF | 0.27 ± 0.09 |
| MAP (mm Hg) | 85 ± 14 |
| NT pro BNP (pg/mL)* | 786 (303-1930) |
| *Renin Angiotensin System parameters* | |
| PRA (ng/mL/h)* | 24.3 (5.5-60.3) |
| Ang II (pmol/L)* | 9.8 (4.2-14.0) |
| *Endothelial function parameters* | |
| UAE (mg/day)* | 8.7 (4.5-21.4) |
| ADMA (umol/L) | 0.68 ± 0.19 |
| vWf (%)* | 78 (48-174) |
| sVCAM-1 (ng/mL)* | 348 (273-374) |
| Plasma NOx (umol/L) | 31.3 ± 15.8 |
| sES (ng/mL)* | 62 (47-88) |
| *Inflammation parameter* | |
| CRP (mg/L) * | 2.5 (1.2-4.3) |

*All continuous variable are presented with mean ± SD.*
*If * is present median value with (25$^{th}$ – 75$^{th}$ percentile) are presented.*
*NYHA; new york heart association functional class, GFR; glomerular filtration rate, RBF; renal blood flow, FF; filtration fraction, LVEF; left ventricular ejection fraction, MAP; mean arterial pressure, NT pro BNP; N terminal pro_brain natriuretic peptide, PRA; plasma renine activity, Ang II; angiotensin II, UAE; urinary excretion rate, ADMA; asymmetric dimethyl arganine, vWF; von Willebrand factor, sVCAM-1; soluble vascular adhesion molecule-1, NOx; nitrate/nitrate, sES; soluble E-selectine, CRP; C-reactive protein.*

## RESULTS

The baseline characteristics of the 86 patients included in this study are presented in Table 1. The studied population consisted predominantly of (76%) male patients with a mean age of 58 ± 12 years. The severity of CHF ranged from NYHA class I to IV with an average of 2.3 ± 0.8. All patients received RAS-inhibition (85% ACE-inhibitor) and the majority was treated with β-blockers (85%) and diuretics (69%). Mean GFR was slightly impaired (74 ml/min/1.73m$^2$) and the proportion of patients with a GFR < 60 ml/min/1.73m$^2$ was 31%. The median UAE was moderately increased (8.7 mg/L) and microalbuminuria (UAE 30-300 mg/day) was present in 19% of the patients.

*Relationships to GFR*

Table 2 shows univariate Pearson and partial correlation coefficients, controlled for age and sex, between GFR and cardiorenal hemodynamic, RAS, endothelium function and inflammation parameters. Table 3 shows the relationships stratified for above and below the median GFR (79.3 mL/min/1.73m$^2$).

RBF was by far the strongest parameter related to GFR, accounting for 85% and 79% of the r$^2$ before and after controlling for age and sex. LVEF was moderately inversely correlated with GFR. NT pro-BNP was strongly inversely correlated with GFR. The relationship with NT pro-BNP was foremost present in the group of patients with a GFR below the median value, whereas it was almost absent in patients with GFR above the median. UAE had an inverse relation with GFR, which was stronger after controlling for age and sex. UAE was especially increased in the group of patients with a renal function below the median GFR.

The relationship with the RAS was assessed using PRA and Ang II. PRA was strongly and inversely correlated with GFR. Ang II was moderately and inversely correlated with GFR and after controlling for age and sex, showing borderline significance ($P$ = 0.07). The relationship between PRA and GFR was present in high and low GFR values, but the relationship of Ang II with GFR was less strong when renal function was more impaired.

The endothelial function markers sVCAM-1, vWf, NOx, ADMA and UAE were inversely associated with renal function at several levels. sES was not related to GFR. When stratified for the level of renal function, sES correlated with GFR in patients with a normal to moderately impaired renal function. The relationships to GFR did not alter significantly when controlled for the baseline age and sex. The majority of the endothelial function markers correlated better in the lower ranges of GFR.

The inflammatory parameter CRP was not related to GFR, even in patients with a normal to moderately impaired renal function.

In Figure 1 the relationships between RBF, GFR, FF and UAE are depicted. As the RBF decreases GFR declines to a similar extent. FF and UAE remain relatively stable along the course of RBF but both parameters alter exponentially when RBF and GFR are seriously reduced (RBF < 275 mL/min/1.73m$^2$ and GFR < 40 mL/min/1.73m$^2$). To fully appreciate the importance of RBF we performed a multivariate analysis in which age and sex and all parameters univariately associated with GFR were included into the model. RBF remained the strongest parameter associated with GFR (74% of the r$^2$). Finally, UAE was not associated with MAP (r = 0.09; $P$ = 0.38) but strongly related with RBF (r = -0.31, $P$ = 0.004).

Table 2. Relation of GFR with conventional cardiorenal hemodynamic, RAS, endothelial and inflammation parameters.

| | GFR | | | |
|---|---|---|---|---|
| Variables | r Unadjusted | *P*-value | r Adjusted for age and sex | *P*-value |
| *Cardiorenal hemodynamic parameters* | | | | |
| RBF | 0.92 | <0.001 | 0.89 | <0.001 |
| UAE# | -0.24 | 0.027 | -0.29 | 0.007 |
| NT pro-BNP# | -0.61 | <0.001 | -0.54 | <0.001 |
| MAP | 0.35 | 0.001 | 0.39 | <0.001 |
| LVEF | 0.22 | 0.043 | 0.29 | 0.006 |
| *Renin Angiotensin System parameters* | | | | |
| PRA# | -0.35 | 0.001 | -0.48 | <0.001 |
| Ang II# | -0.24 | 0.028 | -0.20 | 0.078 |
| *Endothelial function parameters* | | | | |
| sVCAM-1# | -0.30 | 0.006 | -0.29 | 0.007 |
| vWf# | -0.29 | 0.007 | -0.27 | 0.017 |
| NOx | -0.34 | 0.001 | -0.27 | 0.013 |
| ADMA | -0.25 | 0.019 | -0.19 | 0.084 |
| sES# | -0.08 | 0.491 | -0.06 | 0.604 |
| *Inflammation parameter* | | | | |
| CRP# | -0.09 | 0.422 | 0.01 | 0.928 |

*Values are shown as Pearson correlation coefficients and as correlation coefficients obtained with linear regression, adjusted for age and sex. #Data logarithmically transformed.*
*GFR; glomerular filtration rate, RBF; renal blood flow, UAE; urinary excretion rate, NT pro_BNP; N terminal pro_brain natriuretic peptide, MAP; mean arterial pressure, LVEF; left ventricular ejection fraction, PRA; plasma renine activity, Ang II; angiotensin II, sVCAM-1; soluble vascular adhesion molecule-1, vWF; von Willebrand factor, NOx; nitrate/nitrate, ADMA; asymmetric dimethyl arganine, sES; soluble E-selectine, CRP; C-reactive protein.*

### *Other estimates of renal function*

In addition we evaluated whether the relationship between RBF and renal function would be altered, when not GFR was used, but a formula estimating GFR (eGFR) or creatinine clearance (CrCl). The course of various renal function measurements according to decreasing

**Table 3.** Relation of GFR with conventional cardiorenal hemodynamic, RAS, endothelial function and inflamation parameters stratified for median level of GFR.

| | GFR < 79.3 (mL/min/1.73m²) | | GFR > 79.3 (mL/min/1.73m²) | |
|---|---|---|---|---|
| Variables | r | *P*-value | r | *P*-value |
| *Cardiorenal hemodynamic parameters* | | | | |
| RBF | 0.78 | <0.001 | 0.64 | <0.001 |
| UAE# | 0.30 | 0.056 | 0.03 | 0.881 |
| NT pro-BNP | -0.47 | 0.002 | 0.06 | 0.715 |
| MAP | 0.18 | 0.250 | 0.38 | 0.014 |
| LVEF | -0.07 | 0.646 | 0.08 | 0.636 |
| *Renin angiotensin system parameters* | | | | |
| PRA# | -0.43 | 0.006 | -0.35 | 0.029 |
| Ang II# | -0.07 | 0.687 | -0.17 | 0.306 |
| *Endothelial function parameters* | | | | |
| sVCAM-1# | -0.41 | 0.007 | -0.09 | 0.586 |
| vWf | -0.11 | 0.481 | -0.19 | 0.224 |
| NOx | -0.25 | 0.121 | -0.14 | 0.374 |
| ADMA | -0.19 | 0.234 | 0.05 | 0.756 |
| sES# | -0.10 | 0.531 | -0.34 | 0.031 |
| *Inflammation parameter* | | | | |
| CRP | -0.04 | 0.793 | 0.15 | 0.346 |

*Values are shown as correlation coefficients obtained with linear regression, adjusted for age and sex. #Data logarithmically transformed.*
*GFR; glomerular filtration rate, RBF; renal blood flow, UAE; urinary excretion rate, NT pro_BNP; N terminal pro_brain natriuretic peptide, MAP; mean arterial pressure, LVEF; left ventricular ejection fraction, PRA; plasma renine activity, Ang II; angiotensin II, sVCAM-1; soluble vascular adhesion molecule-1, vWF; von Willebrand factor, NOx; nitrate/nitrate, ADMA; asymmetric dimethyl arganine, sES; soluble E-selectine, CRP; C-reactive protein.*

RBF is shown in Figure 2. Both eGFR and CrCl significantly underestimate GFR ($P$ <0.001 and $P$ < 0.001, respectively), but there is a trend toward overestimation at low RBF. Furthermore, at the level where UAE and FF alter exponentially, eGFR and CrCl started to overestimate true GFR.

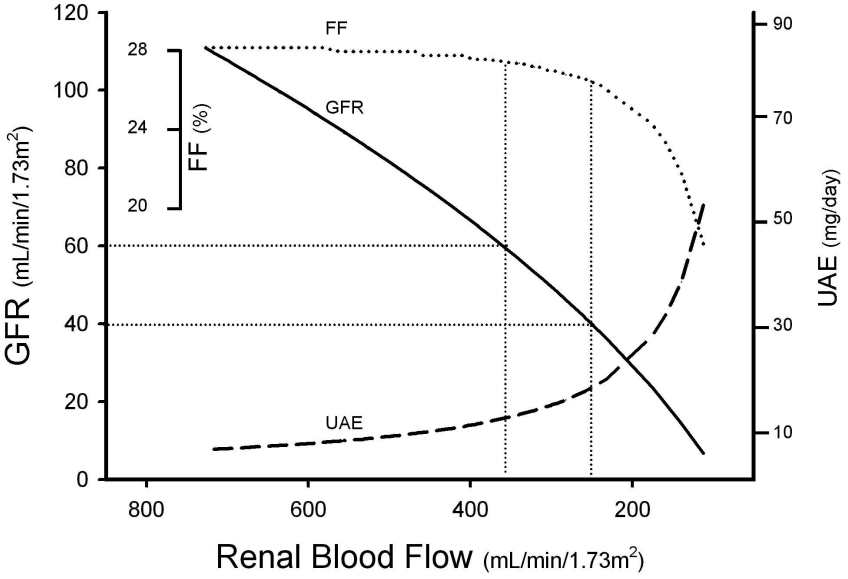


*Figure 1. Relationship between RBF and GFR, FF and UAE in patients with CHF, according to RBF decline.*

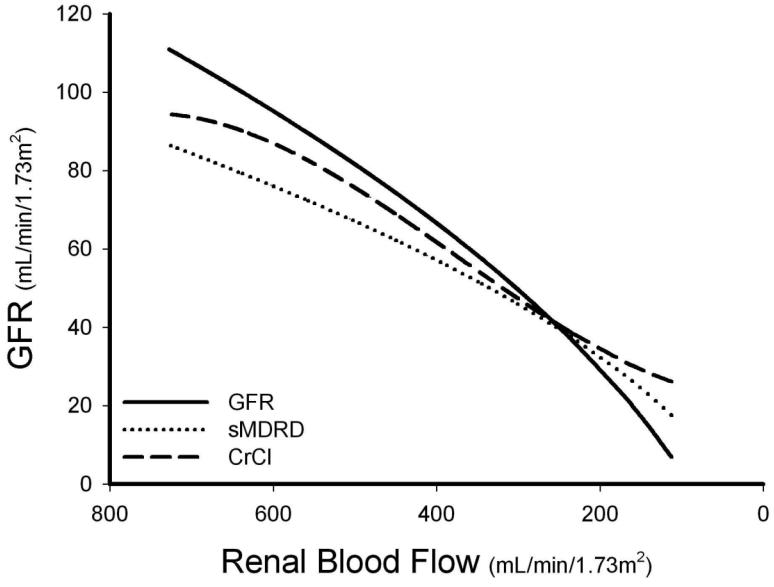


*Figure 2. course of glomerular filtration rate, creatinine clearance and estimated glomerular filtration rate with decreasing renal blood flow*

## DISCUSSION

The present study presents comprehensive data on parameters associated with reduced GFR in patients with CHF. We quantified the relative contribution of the different parameters in the course of renal impairment. We showed that RBF was the key factor in reduced GFR explaining 85% from the observed variance, followed by RAS activation and endothelial dysfunction. Of particular interest is the finding that not only functional but also structural kidney impairment is to be expected for a GFR of less than 40 ml/min per 1.73 $m^2$ of body-surface area – as suggested by the exponential increase of UAE; a range that is shown to be overestimated by the commonly used equations estimating renal function in CHF patients.[15] Clinicians should be aware of the defaults of these methods to estimate renal function necessitating cautious interpretation of the data, especially since these formulas tend to overestimate renal function in the range of structural renal damage.

Renal dysfunction is increasingly recognised to be a major factor related to unfavourable outcome in patients with CHF. The pathophysiology of renal dysfunction in CHF is still incompletely understood. Determination of the factors contributing with renal dysfunction is of key interest to facilitate identification of treatment targets. Previous studies in non-CHF patients, such as patients with diabetes, hypertension or with chronic renal disease suggested links between cardiorenal hemodynamic, the RAS, endothelial function, inflammation parameters and renal function.[7;8;16] These parameters might interact at several levels and eventually result in impaired renal function and structure. Although similar pathways are present in CHF, their influence on renal function can be different.

The single most important determinant of renal function impairment was RBF. In our population 85% of the variance in GFR could be explained by RBF. The impact of RBF on GFR was much larger than observed by Ljungman et al[4] 15 years ago. Recently, we suggested that in CHF reduced GFR is a reflection of impaired hemodynamic status instead of being merely a marker of cardiovascular risk factors or atherosclerosis.[17;18] The almost linear relationship between RBF and GFR in the present study, provides further evidence that in CHF reduced GFR is foremost the result of hemodynamic impairment. Reduced MAP was more strongly related in those with normal to moderately impaired renal function. This finding is at variance with findings in hypertensives, in which renal dysfunction is accompanied with increased MAP, suggesting that reduced GFR in CHF is not related to increased glomerular pressure and ensuing glomerular damage, but rather to reduced hydraulic pressure.[19] Furthermore, we observed that the FF remained stable during moderate renal function impairment and does not increase until GFR drops below approximately 40 ml/min/1.73$m^2$. An increase in FF was suggested to be a possible mechanism to compensate for hypoperfusion of the kidney, based on the data by Ljungman.[4] We assume that the dissociation between hypoperfusion and FF in our study is a consequence of the use of RAS-inhibitors, which currently are imperative in the treatment of CHF.[20] ACE inhibitors show a relatively greater effect in dilating efferent than afferent arterioles, resulting in a decrease in FF. Consequently, glomerular capillary hydraulic pressure will be reduced in proportion to perfusion pressure due to loss of post-glomerular vascular tone, which could be involved in our findings.

Damage and activation of the endothelium play a critical role in the pathogenesis of CHF.[21] We estimated the extent of endothelial damage with several markers and found some were associated with renal function impairment. First, high vWF levels were associated with decreased renal function. vWF is an endothelium derived factor and a well established marker of endothelium dysfunction, which is inversely correlated with flow-mediated endothelial-dependent vasodilatation.[22] Second, we determined plasma NOx levels, which are the resultant of endogenous NO synthesis, oxidative stress and urinary NOx excretion and therefore should be interpreted with caution. We found that increased NOx levels were associated with decreased renal function. Increased NOx levels have also been associated with heart failure and other conditions characterised by endothelial dysfunction, such as hypertension and hyperlipidaemia.[23-25]

Third, in addition to NOx, we determined ADMA levels, an endogenous NO synthase inhibitor. Previously, elevated ADMA levels were reported in cardiovascular patients and in patients with end-stage renal disease.[26;27] In patients with renal disease ADMA predicted progression of renal dysfunction and mortality.[28;29] Only recently Kielstein et al[30], showed that ADMA was an important substance influencing renal perfusion. We now report that increased ADMA levels are associated with mild-renal function impairment in patients with CHF. However, this relation was mainly driven by patients with the most affected renal function. Finally, we determined inflammatory activation of the endothelium by sES and VCAM-1 levels. These soluble forms of endothelial leukocyte adhesion molecules are considered to be surrogate markers of their true endothelial expression. Adhesion molecule-regulated leukocyte recruitment can result in functional impairment of the endothelium. In our patients only VCAM-1 was associated with renal function impairment. This might be explained by their differential roles in leukocyte adhesion. sES predominantly mediates the initial, low-affinity leukocyte-endothelial cell interaction while VCAM-1 mediates the subsequent firm adhesion and transendothelial migration of leukocytes, resulting in infiltration of leukocytes below the endothelial layer.[31;32]

Inflammation is recognised as an important marker for future cardiovascular events.[33] We determined high-sensitive CRP as a marker of systemic inflammation. Although median value was well above the value of 2 mg/L needed to maximize patient benefit, we did not observe an association between CRP and renal function impairment. This suggests that the decreased survival associated with renal dysfunction might be independent of CRP levels. This might explain why statin treatment significantly reduces CRP, but only has a modest effect on kidney function loss.[34]

In our CHF population UAE was inversely related to GFR. However, this relation was mainly driven by a marked increase in UAE in the patients with severely impaired renal function. The exponential increase of UAE as GFR deteriorates below 40 ml/min/1.73m$^2$ may imply a certain threshold below which the kidney is unable to maintain its structural integrity, as a consequence of renal hypoperfusion. In non-CHF conditions, UAE is related to increased MAP[35], indicating gradual glomerular damage to increased intraglomerular pressure. In our patients MAP was not associated with increased UAE, instead the most direct marker of renal hypoperfusion, RBF, was strongly related to UAE. Under chronic conditions, intrarenal hypoperfusion injury can progress to compromise the entire kidney.[36]

Chronic renal hypoperfusion might ultimately result in damage of the tubulus. The tubulus is known to reabsorb albumin and tubular damage will therefore increase UAE. Therefore, these results might imply that increased UAE in CHF is the result of tubular damage due to chronic hypoperfusion. The higher UAE could also be considered to reflect more extensive atherosclerosis. However, it was also present in subjects with non-ischemic, i.e non-atherosclerotic heart failure, which renders it less likely that UAE in this population merely reflects the severity of underlying atherosclerosis. The elevated UAE was found in spite of treatment with RAS-blockade, which is known to reduce UAE.

Finally, we studied whether the relationship between RBF and renal function would be altered if not true GFR was used, but the sMDRD formula, which is commonly used to estimate GFR or creatinine clearance (CrCl). Both the sMDRD and CrCl significantly underestimate GFR. Importantly, there is a trend toward overestimation at lower RBF. Considering the observation that UAE develops in the very low ranges of true GFR clinicians should be very cautious relying on these formulas, overestimating renal function.

*Limitations*

This study is of cross-sectional design and thus only can be hypothesis generating. Regretfully we were not able to determine the relationship of the sympathetic nervous system and renal function in our patients. In CHF the sympathetic nervous system might also play an important role in renal function impairment. However, to our knowledge this is the largest cohort of patients comparing the results of true renal function measurements with a large number of parameters linked to the biology of impaired renal function. Only a few, small sized studies using limited numbers of parameters are available.[30;37]

## CONCLUSIONS

Our observations suggest an interrelationship between renal hypoperfusion, RAS activation, endothelial dysfunction, inflammation, on the one hand and renal injury on the other hand in patients with CHF treated with RAS-blockade. This results in a vicious circle of potentially irreversible renal damage. These factors might be mediators involved in the increased cardiovascular risk observed in CHF patients with impaired renal function. In CHF patients with seriously impaired renal function, UAE is exponentially increased, suggesting structural damage to the kidney, possibly due to chronic hypoperfusion.

**Reference List**

1. Hillege HL, Girbes AR, de Kam PJ, Boomsma F, de Zeeuw D, Charlesworth A, Hampton JR, van Veldhuisen DJ. Renal function, neurohormonal activation, and survival in patients with chronic heart failure. *Circulation*. 2000;102:203-210.

2. Smilde TD, Hillege HL, Voors AA, Dunselman PH, van Veldhuisen DJ. Prognostic importance of renal function in patients with early heart failure and mild left ventricular dysfunction. *Am J Cardiol*. 2004;94:240-243.

3. Leithe ME, Margorien RD, Hermiller JB, Unverferth DV, Leier CV. Relationship between central hemodynamics and regional blood flow in normal subjects and in patients with congestive heart failure. *Circulation*. 1984;69:57-64.

4. Ljungman S, Lausch RN, Cody R. Role of the kidney in congestive heart failure. Relationship of cardiac index to kidney function. *Drugs*. 1990;39:discussion 22-24.

5. Culleton BF, Larson MG, Wilson PW, Evans JC, Parfrey PS, Levy D. Cardiovascular disease and mortality in a community-based cohort with mild renal insufficiency. *Kidney Int*. 1999;56:2214-2219.

6. Manjunath G, Tighiouart H, Ibrahim H, MacLeod B, Salem DN, Griffith JL, Coresh J, Levey AS, Sarnak MJ. Level of kidney function as a risk factor for atherosclerotic cardiovascular outcomes in the community. *J Am Coll Cardiol*. 2003;41:47-55.

7. Perticone F, Maio R, Tripepi G, Zoccali C. Endothelial Dysfunction and Mild Renal Insufficiency in Essential Hypertension. *Circulation*. 2004.

8. Stam F, van Guldener C, Schalkwijk CG, ter Wee PM, Donker AJ, Stehouwer CD. Impaired renal function is associated with markers of endothelial dysfunction and increased inflammatory activity. *Nephrol Dial Transplant*. 2003;18:892-898.

9. Palatini P, Mormino P, Mos L, Mazzer A, Dorigatti F, Zanata G, Longo D, Garbelotto R, De Toni R, Graniero G, Pessina AC. Microalbuminuria, renal function and development of sustained hypertension: a longitudinal study in the early stage of hypertension. *J Hypertens*. 2005;23:175-182.

10. Stehouwer CD, Nauta JJ, Zeldenrust GC, Hackeng WH, Donker AJ, den Ottolander GJ. Urinary albumin excretion, cardiovascular disease, and endothelial dysfunction in non-insulin-dependent diabetes mellitus. *Lancet*. 1992;340:319-323.

11. Hillege HL, Fidler V, Diercks GF, van Gilst WH, de Zeeuw D, van Veldhuisen DJ, Gans RO, Janssen WM, Grobbee DE, de Jong PE. Urinary albumin excretion predicts cardiovascular and noncardiovascular mortality in general population. *Circulation*. 2002;106:1777-1782.

12. Donker AJ, van der Hem GK, Sluiter WJ, Beekhuis H. A radioisotope method for simultaneous determination of the glomerular filtration rate and the effective renal plasma flow. *Neth J Med*. 1977;20:97-103.

13. Manjunath G, Sarnak MJ, Levey AS. Prediction equations to estimate glomerular filtration rate: an update. *Curr Opin Nephrol Hypertens*. 2001;10:785-792.

14. de Leeuw K, Sanders JS, Stegeman C, Smit A, Kallenberg CG, Bijl M. Accelerated atherosclerosis in patients with Wegener's granulomatosis. *Ann Rheum Dis*. 2005;64:753-759.

15. Smilde TD, van Veldhuisen DJ, Navis G, Voors AA, Hillege H. Formulas estimating renal function are inaccurate and biased in patients with chronic heart failure. *Submitted*. 2005.

16. Jacobson SH, Egberg N, Hylander B, Lundahl J. Correlation between soluble markers of endothelial dysfunction in patients with renal failure. *Am J Nephrol*. 2002;22:42-47.

17. Smilde TD, Hillege HL, Navis G, Boomsma F, de Zeeuw D, van Veldhuisen DJ. Impaired renal function in patients with ischemic and nonischemic chronic heart failure: association with neurohormonal activation and survival. *Am Heart J*. 2004;148:165-172.

18. Smilde TD, Hillege H, Navis G, Voors AA, Brouwer J, Veldhuisen van DJ. Difference in long-term prognostic value of renal function between ischemic and non-ischemic mild heart failure. *Int J Cardiol*. 2005;In press.

19. Ruilope LM, Campo C, Rodicio JL. Relationship between blood pressure and renal function. *J Hypertens Suppl*. 1994;12:S55-S59.

20. Swedberg K, Cleland J, Dargie H, Drexler H, Follath F, Komajda M, Tavazzi L, Smiseth OA, Gavazzi A, Haverich A, Hoes A, Jaarsma T, Korewicki J, Levy S, Linde C, Lopez-Sendon JL, Nieminen MS, Pierard L, Remme WJ. Guidelines for the diagnosis and treatment of chronic heart failure: executive summary (update 2005): The Task Force for the Diagnosis and Treatment of Chronic Heart Failure of the European Society of Cardiology. *Eur Heart J*. 2005;26:1115-1140.

21. Fischer D, Rossa S, Landmesser U, Spiekermann S, Engberding N, Hornig B, Drexler H. Endothelial dysfunction in patients with chronic heart failure is independently associated with increased incidence of hospitalization, cardiac transplantation, or death. *Eur Heart J*. 2005;26:65-69.

22. Chong AY, Blann AD, Patel J, Freestone B, Hughes E, Lip GY. Endothelial dysfunction and damage in congestive heart failure: relation of flow-mediated dilation to circulating endothelial cells, plasma indexes of endothelial damage, and brain natriuretic peptide. *Circulation*. 2004;110:1794-1798.

23. Shibata H, Nabika T, Moriyama H, Masuda J, Kobayashi S. Correlation of NO metabolites and 8-iso-prostaglandin F2a with periventricular hyperintensity severity. *Arterioscler Thromb Vasc Biol*. 2004;24:1659-1663.

24. Takahashi H, Nakanishi T, Nishimura M, Tanaka H, Yoshimura M. Measurements of serum levels of nitrate ions in men and women: implications of endothelium-derived relaxing factor in blood pressure regulation and atherosclerosis. *J Cardiovasc Pharmacol*. 1992;20 Suppl 12:S214-S216.

25. Winlaw DS, Smythe GA, Keogh AM, Schyvens CG, Spratt PM, Macdonald PS. Increased nitric oxide production in heart failure. *Lancet*. 1994;344:373-374.

26. Valkonen VP, Paiva H, Salonen JT, Lakka TA, Lehtimaki T, Laakso J, Laaksonen R. Risk of acute coronary events and serum concentration of asymmetrical dimethylarginine. *Lancet*. 2001;358:2127-2128.

27. Zoccali C, Bode-Boger S, Mallamaci F, Benedetto F, Tripepi G, Malatino L, Cataliotti A, Bellanuova I, Fermo I, Frolich J, Boger R. Plasma concentration of asymmetrical dimethylarginine and mortality in patients with end-stage renal disease: a prospective study. *Lancet*. 2001;358:2113-2117.

28. Ravani P, Tripepi G, Malberti F, Testa S, Mallamaci F, Zoccali C. Asymmetrical dimethylarginine predicts progression to dialysis and death in patients with chronic kidney disease: a competing risks modeling approach. *J Am Soc Nephrol*. 2005;16:2449-2455.

29. Fliser D, Kronenberg F, Kielstein JT, Morath C, Bode-Boger SM, Haller H, Ritz E. Asymmetric dimethylarginine and progression of chronic kidney disease: the mild to moderate kidney disease study. *J Am Soc Nephrol*. 2005;16:2456-2461.

30. Kielstein JT, Bode-Boger SM, Klein G, Graf S, Haller H, Fliser D. Endogenous nitric oxide synthase inhibitors and renal perfusion in patients with heart failure. *Eur J Clin Invest*. 2003;33:370-375.

31. Blake GJ, Ridker PM. Novel clinical markers of vascular wall inflammation. *Circ Res*. 2001;89:763-771.

32. Meigs JB, Hu FB, Rifai N, Manson JE. Biomarkers of endothelial dysfunction and risk of type 2 diabetes mellitus. *JAMA*. 2004;291:1978-1986.

33. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med*. 2000;342:836-843.

34. Tonelli M, Isles C, Craven T, Tonkin A, Pfeffer MA, Shepherd J, Sacks FM, Furberg C, Cobbe SM, Simes J, West M, Packard C, Curhan GC. Effect of pravastatin on rate of kidney function loss in people with or at risk for coronary disease. *Circulation*. 2005;112:171-178.

35. Stuveling EM, Bakker SJ, Hillege HL, Burgerhof JG, de Jong PE, Gans RO, de Zeeuw D. C-reactive protein modifies the relationship between blood pressure and microalbuminuria. *Hypertension*. 2004;43:791-796.

36. Brezis M, Rosen S. Hypoxia of the renal medulla—its implications for disease. *N Engl J Med*. 1995;332:647-655.

37. Cody RJ, Ljungman S, Covit AB, Kubo SH, Sealey JE, Pondolfino K, Clark M, James G, Laragh JH. Regulation of glomerular filtration rate in chronic congestive heart failure patients. *Kidney Int*. 1988;34:361-367.