



Published in final edited form as:

Am J Nephrol. 2014 ; 39(6): 543–552. doi:10.1159/000363584.

A Pilot Study to Evaluate Renal Hemodynamics in Cirrhosis by Simultaneous Glomerular Filtration Rate, Renal Plasma Flow, Renal Resistive Indices and Biomarkers Measurements

Ayşe L. Mindikoglu, M.D., M.P.H.¹, Thomas C. Dowling, Pharm.D., Ph.D.², Jade J. Wong-You-Cheong, MBChB³, Robert H. Christenson, Ph.D.⁴, Laurence S. Magder, Ph.D. M.P.H.⁵, William R. Hutson, M.D.¹, Stephen L. Seliger, M.D., M.S.⁶, and Matthew R. Weir, M.D.⁶

¹Department of Medicine, Division of Gastroenterology and Hepatology, University of Maryland School of Medicine

² University of Maryland School of Pharmacy

³Department of Diagnostic Radiology and Nuclear Medicine, University of Maryland School of Medicine

⁴Department of Pathology, University of Maryland School of Medicine

⁵Department of Epidemiology and Public Health, Division of Biostatistics and Bioinformatics, University of Maryland School of Medicine

⁶Department of Medicine, Division of Nephrology, University of Maryland School of Medicine; University of Maryland School of Medicine

Abstract

Background—Renal hemodynamic measurements are complicated to perform in patients with cirrhosis; yet they provide the best measure of risk to predict hepatorenal syndrome (HRS). Currently, there are no established biomarkers of altered renal hemodynamics in cirrhosis validated by measured renal hemodynamics.

Methods—In this pilot study, simultaneous measurements of glomerular filtration rate (GFR), renal plasma flow (RPF), renal resistive indices and biomarkers were performed to evaluate renal hemodynamic alterations in 10 patients with cirrhosis (3 patients without ascites, 5 with diuretic sensitive and 2 diuretic refractory ascites).

Results—Patients with diuretic refractory ascites had the lowest mean GFR (36.5 ml/min/1.73m²) and RPF (133.6 ml/min/1.73m²) when compared to those without ascites (GFR=82.9 ml/min/1.73m², RPF=229.9 ml/min/1.73m²) and with diuretic-sensitive ascites (GFR=82.3 ml/min/1.73m², RPF=344.1 ml/min/1.73m²). A higher mean filtration fraction (FF= GFR/RPF=0.36) was noted among those without ascites compared to those with ascites. Higher FF in

CORRESPONDANCE: Ayşe L. Mindikoglu, M.D., M.P.H. Assistant Professor of Medicine Division of Gastroenterology and Hepatology University of Maryland School of Medicine 22 S. Greene Street, N3W50 Baltimore, Maryland 21201 Phone: 410 328 1358 Fax: 410 328 1897 amindiko@medicine.umaryland.edu.

CONFLICT OF INTEREST STATEMENT

None of the authors has conflict of interest associated with the manuscript.

patients without ascites is most likely secondary to the vasoconstriction in the efferent glomerular arterioles (normal FF \approx 0.20). In general, renal resistive indices were inversely related to FF. While patients with ascites had lower FF and higher right kidney main and arcuate artery resistive indices, those without ascites had higher FF and lower right kidney main and arcuate artery resistive indices. While cystatin C and beta-2 microglobulin performed better compared to Cr in estimating RPF; beta-trace protein, beta-2 microglobulin, SDMA, and (SDMA+ADMA) performed better in estimating right kidney arcuate artery resistive index.

Conclusion—The results of this pilot study showed that identification of non-invasive biomarkers of reduced RPF and increased renal resistive indices can identify cirrhotics at risk for HRS at a stage more amenable to therapeutic intervention, and reduce mortality from kidney failure in cirrhosis.

Keywords

End-stage liver disease; glomerular filtration rate; renal plasma flow; renal resistive index; cystatin C; beta-trace protein; beta-2 microglobulin; kidney injury molecule-1 (KIM-1); Neutrophil Gelatinase-Associated Lipoprotein (NGAL)

INTRODUCTION

The estimated prevalence of acute and chronic kidney diseases among hospitalized patients with cirrhosis is reported to be 20%¹. Acute kidney injury (AKI) in cirrhosis is associated with high morbidity and mortality²⁻⁴. In particular, hepatorenal syndrome (HRS), a specific cause of AKI⁵ due to severe reduction of renal blood flow^{1,4,6-9} and may lead to consequent acute tubular necrosis (ATN) requiring renal replacement therapy. Without liver and possibly simultaneous liver-kidney transplantation, HRS is frequently fatal^{1,4,6}. Based on Organ Procurement and Transplantation Network data as of February 21, 2014; 8% of all adult liver-transplants performed in 2012 were simultaneous liver-kidney transplants (excluding simultaneous liver-kidney heart, liver-kidney intestine, liver-kidney pancreas, liver-kidney-pancreas-intestine)¹⁰.

Current treatments of HRS include splanchnic vasoconstrictors (Octreotide, Terlipressin), alpha-agonists and albumin. These treatments are effective in fewer than 50% of patients with cirrhosis^{3,4}. The lack of efficacy in the treatment of HRS is likely due to delays in the administration of splanchnic vasoconstrictors caused by the limitation of the current diagnostic criteria of HRS¹¹. There is no gold standard method to diagnose HRS; it is largely a diagnosis of exclusion. It can also be superimposed on anatomical kidney diseases, or functional changes in renal function such as prerenal azotemia due to gastrointestinal bleeding, further complicating the diagnosis. Existing methods to identify reduced renal blood flow in cirrhosis that are clinically practical have limited accuracy¹¹. First, serum creatinine (Cr) is not a sufficiently accurate marker of kidney function in cirrhosis¹²; yet current diagnostic criteria for HRS is based on serum Cr. Furthermore, reduction in renal blood flow, particularly renal cortical blood flow, is a key phenomenon in the development of HRS and may precede the increase in Cr and decline in glomerular filtration rate (GFR)¹³. Second, current criteria¹⁴ are inadequate to diagnose HRS in subjects with cirrhosis and underlying chronic kidney disease (CKD) who already have elevated Cr

levels^{5,11}. Third, these criteria traditionally require two days of albumin administration to differentiate hypovolemia-induced prerenal azotemia from HRS¹⁴. Waiting for two days to determine the effects of albumin infusion can cause substantial delays in initiation of vasoconstrictor treatment in HRS and reduce the efficacy of existing treatments¹¹. Differentiation of prerenal azotemia from HRS may not be the best strategy, as cirrhotics can have combined HRS and hypovolemia-induced azotemia¹¹.

Currently, there are no established biomarkers of altered renal hemodynamics in cirrhosis validated by measured GFR and renal plasma flow (RPF) measurements. The primary objective of this pilot study was to assess renal hemodynamic levels in different stages of cirrhosis using a gold-standard invasive assessment and determine whether novel non-invasive biomarkers including cystatin C, beta-trace protein, beta-2 microglobulin and dimethylarginines were more accurate estimators of altered renal hemodynamics compared to Cr in patients with cirrhosis.

METHODS

Study Participants

Between September and December 2013, patients with cirrhosis were recruited from the University of Maryland Medical Center. Inclusion criteria were having cirrhosis based on either liver histopathology or clinical, laboratory and radiological results and being 18 years of age or older at the time of enrollment. Exclusion criteria included inability to give informed consent and cognitive impairment; being pregnant or breastfeeding; allergy to iohalamate, iodine, iodine-containing radiographic contrast media or para-aminohippurate (PAH); untreated active hyperthyroidism, thyroid autonomy, multinodular goiter, or ongoing treatment with radioactive iodine; limitations for voiding or collecting urine; renal failure treated with dialysis or estimated GFR < 15 ml/min/1.73m²; treatment with corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs) except any daily dose of aspirin lower than 325 mg within 1 week of enrollment; any change in the dose of diuretics, angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers or new onset of diuretic, angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers use within 1 week of enrollment; acute onset of infection, hospitalization due to exacerbation of encephalopathy, acute gastrointestinal bleeding, acute kidney injury within 1 week of enrollment; acute cardiovascular or cerebrovascular disease diagnosed within 3 weeks of enrollment; history of transjugular intrahepatic porto-systemic shunt placement, and previous kidney or liver transplantation.

The study was approved by the Institutional Review Board of the University of Maryland, Baltimore, and all study subjects provided written informed consent.

Study Visits and Procedures

The study included a screening visit that was conducted either at the University of Maryland Medical Center clinics or General Clinical Research Center (GCRC) and a procedure visit that was conducted at the GCRC. During screening visit, subjects were enrolled in the study and had a complete history and physical examination taken. They were instructed to have an

overnight fast prior to the procedure day except they were allowed to take water and medications. If they were diabetic, they were asked to fast only for 2 hours.

The 6-hour procedure visit was scheduled in GCRC within 1 to 3 weeks of screening visit. On the procedure date, after consuming 500 ml of water, subjects underwent simultaneous measurements of RPF, GFR, renal resistive indices and biomarkers. The procedures and methods were as follows:

Renal Plasma Flow (RPF) Measurement—RPF was measured by PAH clearance as described in Wang *et al.*¹⁵. After an IV loading dose of 200 mg (1 mL), subjects received a constant infusion to achieve steady-state PAH plasma concentration of 20 mg/L. All subjects received PAH infusion at 60 mL/hr. The desired maintenance doses were based on the subjects' screening GFR estimated by Modification of Diet in Renal Disease (MDRD) Study equation¹⁶. A 5 ml of blood sample was collected in heparinized tubes before and at 5, 15, 30, 45, 60, 120, 180, 240 and 360 min after the initiation of PAH administration. Following a pre-PAH urine collection, spontaneous urine collections were obtained at 0-60, 60-120, 120-180, and 180-360 min after starting the PAH infusion and urine volume collected at each time point was measured and 3 ml was transferred into a test tube. Plasma and urine samples were harvested by centrifugation, and aliquots stored at -20°C until analysis. Plasma and urine PAH concentrations were determined using High Performance Liquid Chromatography (HPLC) assay method¹⁷. RPF was calculated as the average renal clearance of PAH during the 360 min urine collection period as Renal PAH Clearance = (Amount of PAH excreted)_{x-y} / (Area under the plasma PAH concentration vs. time curve)_{x-y}, where x-y was the time interval of the urine collection period.

GFR Measurement—GFR was measured by non radiolabeled iothalamate plasma clearance as described in Mindikoglu *et al.*¹⁸. The same blood samples as collected for RPF measurements were used for the GFR measurements. Plasma concentrations of iothalamate were determined by reversed-phase HPLC with ultraviolet (UV) detection as described by Dowling *et al.*¹⁷. Plasma iothalamate concentration *versus* time curve was modeled based on two-compartment model¹⁹ applying iterative least-square method using WinNonlin[®] version 5.1 (Certara L.P. (Pharsight), St. Louis, MO).

Measurement of Renal Resistive Indices.—Duplex Doppler Ultrasonography of both kidneys was performed using the Philips Healthcare iU22 Ultrasound System with C5-1 convex transducer. Duplex waveforms of the main, segmental, interlobar and arcuate arteries were assessed in the upper, mid and lower poles in both kidneys and peak systolic velocity, end diastolic velocity and resistive indices were measured simultaneously with GFR and RPF measurements. Renal resistive index was calculated as [(peak systolic velocity-end diastolic velocity)/peak systolic velocity]²⁰. Two repeated measurements were taken from each renal artery; renal resistive index of each artery was reported as the mean value of these two measurements. Final resistive index was reported as the mean value of the mean resistive indices of the upper, mid and lower segmental, interlobar and arcuate arteries (e.g. arcuate artery resistive index reported in the analysis was equal to the mean value of the mean resistive indices of the upper, mid and lower arcuate arteries). Duplex Doppler Ultrasonography of all patients was performed by the same sonographer.

Measurement of Systemic Hemodynamics—Subjects' intravascular volume status was assessed by measuring inferior vena cava (IVC) diameter during expiration using ultrasonography simultaneously with GFR and RPF measurements. Three measurements were taken from the IVC; IVC diameter was reported as the mean value of these three measurements.

Renal Hemodynamic Biomarkers—Pre-RPF/GFR blood samples were collected for symmetric (SDMA) and asymmetric dimethylarginine (ADMA), L-arginine, renin and aldosterone levels. SDMA, ADMA, L-arginine were measured using Diagnostika® standard ELISA method^{21,22}, renin using Renin Human ELISA method (BioVendor Research and Diagnostic Products)²³ and aldosterone using ELISA method (BioVendor Research and Diagnostic Products)²⁴.

Filtration Markers: Pre-RPF/GFR blood samples were collected for Cr, cystatin C, beta-trace protein, beta 2 microglobulin measurements. Cr, cystatin C and beta-2 microglobulin concentrations were measured using Siemens Dimension Vista® System Flex® reagent cartridge (Siemens Healthcare Diagnostics Inc, Newark, DE)^{25,27}. Beta-trace protein was measured using N Latex β TP assay using Siemens ProSpec® nephelometer (Siemens Healthcare Diagnostics Inc, Newark, DE)²⁸ at the University of Minnesota Advanced Research and Diagnostic Laboratory. *Other Laboratory Tests.* Pre-RPF/GFR blood samples were collected for basic metabolic panel, complete blood count with differential, hepatic panel consisting of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total and direct bilirubin, alkaline phosphatase (ALP), albumin, globulin, and total protein; prothrombin time (PT)/international normalized ratio (INR) and C reactive protein.

Urine Tests: Pre-RPF/GFR urine samples were collected for microscopic analysis, Cr and protein to calculate spot urine protein to Cr ratio, kidney injury molecule-1 (KIM-1) and Neutrophil Gelatinase-Associated Lipoprotein (NGAL). NGAL was measured using Quantikine® ELISA Human Lipocalin 2/NGAL Immunoassay (R&D Systems, Inc)²⁹ and KIM-1 using Human KIM 1 ELISA Kit (Aviscera Bioscience, Inc)³⁰.

Statistical Analysis

For statistical analyses, we used SAS, Version 9.2 (Cary, NC)³¹ and Minitab (Minitab, Inc., State College, PA)³² statistical softwares. Linear regression analysis was performed to assess the performance of renal biomarkers to predict RPF and renal arcuate artery resistive index.

RESULTS

Study Participants

A total of 14 subjects with cirrhosis were enrolled in the study; 10 out of 14 (7 men and 3 women; age ranging from 45 to 69) completed all study procedures. **Table 1** shows clinical and laboratory characteristics of 10 patients with cirrhosis. Seven patients had hepatitis C cirrhosis and 3 alcohol cirrhosis. Three patients had no ascites, 5 diuretic sensitive ascites and 2 diuretic-refractory ascites. While the lowest Model for End-Stage Liver Disease

(MELD) score was 8, the highest was 19. All patients except one (spot urine protein to Cr ratio=0.25) had a spot urine protein to Cr ratio lower than 0.2 suggesting no evidence for clinically significant glomerular disease. No patient's urine KIM-1 and NGAL level was greater than the optimal cut points of KIM-1 (15.4 ng/ml=15,400 pg/ml) and NGAL (365 ng/ml) that differentiates ATN from non-ATN as described by Belcher *et al.*³³

Renal Hemodynamics

Table 2 shows GFR and RPF measured simultaneously with renal resistive indices of main, segmental, interlobar and arcuate arteries of both kidneys. No subjects had evidence of volume depletion or overload; mean IVC diameter was 1.61 cm, ranging from 1.07 cm to 2.19 cm. In general, FF (GFR/RPF) was lower and renal resistive indices were higher in patients with ascites compared to those without ascites (**Table 3**). A higher mean FF (0.36) was noted among those without ascites compared to those with ascites (**Table 3**). Patients with diuretic-refractory ascites had the lowest mean GFR (36.5 ml/min/1.73m²) and mean RPF (133.6 ml/min/1.73m²) when compared to those without ascites (GFR=82.9 ml/min/1.73m², RPF=229.9 ml/min/1.73m²) and with diuretic-sensitive ascites (82.3 ml/min/1.73m², 344.1 ml/min/1.73m²) (**Table 3**).

Renal Resistive Indices

As shown in **Table 3**, mean renal resistive indices were higher in cirrhotics with ascites compared to those without ascites. In general, renal resistive indices, particularly right kidney arcuate artery resistive indices showed medium level negative correlation with FF (Pearson correlation coefficient $r = -0.44$, $P = 0.20$) (**Figure 1**); while patients with ascites had lower FF and higher right kidney main and arcuate artery resistive indices, those without ascites had higher FF and lower right kidney main and arcuate artery resistive indices (**Figures 2, 3 and 4**). We observed similar findings with left kidney main and arcuate artery resistive indices.

Biomarkers

Additionally, we measured renal biomarkers including serum Cr, cystatin C, beta-trace protein, beta-2 microglobulin, SDMA, ADMA and L-arginine simultaneously with GFR, RPF and renal resistive indices.

In terms of estimating RPF, the linear regression fit was better for cystatin C (R-Square=0.43, $P = 0.038$), beta-trace protein (R-Square=0.35, $P = 0.073$) and beta-2 microglobulin (R-Square=0.46, $P = 0.030$) compared to Cr (R-Square=0.31, $P = 0.094$), SDMA (R-Square=0.27, $P = 0.124$), the sum of SDMA and ADMA (SDMA+ADMA) (R-Square=0.24, 0.155), L-arginine (R Square=0.03, $P = 0.643$), ADMA (R-Square=0.15, $P = 0.267$) and L-arginine/SDMA ratio (R-Square=0.20, $P = 0.199$) (**Figure 5**). We obtained similar results when we controlled for age, sex and race.

While all biomarkers appeared to perform better compared to serum Cr (R-Square=0.20, $P = 0.198$) in estimating renal cortical blood flow assessed by right kidney arcuate artery resistive index; the beta-trace protein (R-Square=0.52, $P = 0.018$), beta 2 microglobulin (R-Square=0.42, $P = 0.043$), SDMA (R-Square=0.44, $P = 0.038$), (SDMA+ADMA) (R-

Square=0.42, P=0.044) performed the best (**Figure 6**). The performance of L-arginine (R-Square=0.04, P=0.585), ADMA (R-Square=0.31, P=0.092) and L-arginine/SDMA ratio (R-Square=0.05, P=0.537) was poor.

DISCUSSION

In this pilot study, we performed simultaneous measurements of GFR, RPF, renal resistive indices and biomarkers to evaluate patterns of change in different stages of cirrhosis. To our knowledge, our study is the first to measure renal resistive indices simultaneously with direct RPF and GFR measurements in cirrhotics. Our results suggest that in different stages of cirrhosis assessed by the type of ascites (no ascites, diuretic sensitive and –refractory ascites), there are distinct patterns of glomerular hemodynamics and resistive indices.

We observed a higher mean FF (FF=GFR/RPF=0.36) among those without ascites compared to those with ascites (**Table 3**). Higher FF in patients without ascites is most likely secondary to the vasoconstriction in the efferent glomerular arterioles (normal FF≈0.20). Among those with diuretic-refractory ascites, we observed the lowest GFR and RPF consistent with the results of our recent study¹⁸. Among those with ascites, FF was near normal and renal resistive indices were higher. In general, renal resistive indices were inversely related to FF (**Figure 1**); while patients with ascites had lower FF and higher right kidney main and arcuate artery resistive indices, those without ascites had higher FF and lower kidney main and arcuate artery resistive indices (**Figures 2, 3 and 4**). These findings suggest that in late stages of cirrhosis, reduced RPF is associated with increase in renal arterial resistive indices as there is no compensatory increase in FF. According Poiseuille's law³⁴, resistance to flow is inversely related to volume flow rate. As renal vascular resistance is positively and significantly correlated with renal resistive indices³⁵, our findings of reduced RPF with increased renal resistive indices suggest that our findings are in line with Poiseuille's law. While cystatin C and beta-2 microglobulin performed better compared to Cr in estimating RPF (Figure 5); beta-trace protein, beta-2 microglobulin, SDMA, and (SDMA+ADMA) performed better in estimating right kidney arcuate artery resistive index (Figure 6).

In our recent review, we emphasized the importance of simultaneous evaluation of GFR, RPF and FF in cirrhosis and proposed a dynamic classification system that takes both GFR and RPF into account in evaluating kidney dysfunction in cirrhosis¹¹. In 1951, Leslie *et al.*³⁶ evaluated renal hemodynamics in 17 patients with cirrhosis and showed the importance of FF (simultaneous GFR and RPF measurements) in cirrhosis. In patients without ascites, mean FF was elevated; whereas in those with ascites, mean FF was normal³⁶. The elevation in FF most likely resulted from the vasoconstrictive effect of angiotensin II on efferent glomerular arterioles^{11,36,37}. On the other hand, simultaneous reductions in mean GFR and RPF resulted in normal/subnormal levels of FF in parallel to the severity of ascites. The results of our study are in line with these findings (**Table 3**).

Additionally, we showed that normal FF (FF≈0.20) was associated with reductions in renal cortical blood flow that was assessed by renal arcuate artery resistive indices (**Figure 1**). Arcuate arteries are located at the junction of renal cortex and medulla and give rise to

interlobular arteries that supply the renal cortex³⁸. Measurement of interlobular artery resistive indices by Duplex Doppler ultrasound is technically challenging and poorly reproducible in patients with cirrhosis and ascites given the size of the arteries^{39,40}. Therefore, we elected to measure resistive indices in the arcuate arteries to assess the renal cortical blood flow. The increase in resistive indices in the right kidney in patients with decompensated cirrhosis, particularly in those with diuretic refractory ascites was previously shown by Rivolta *et al.*⁴¹. However, the authors did not validate increases in renal resistive indices by simultaneous RPF measurements; rather they compared alterations in renal resistive indices with Cr clearance which is not an accurate measure of GFR in cirrhosis⁴¹. In this study, we confirmed the increases in renal resistive indices by the decrease in RPF measured by PAH clearance.

Our study suggests that both normal FF (≈ 0.20) and an increase in renal resistive indices can be associated with severe reductions in renal blood flow in cirrhosis. Development of spontaneous bacterial peritonitis, sepsis, aggressive diuresis and frequent paracenteses at this stage may increase risk of patients with cirrhosis to develop ATN in the setting of already severely reduced renal blood flow. Therefore, early identification of this stage by noninvasive biomarkers may facilitate early management in patients with cirrhosis including holding diuretics, infusion of albumin, and administration of vasoconstrictor drugs before waiting for increases in Cr.

Our study was limited to only 10 patients with cirrhosis which precluded formal statistical inference testing of between-group differences and correlations of RPF with the circulating biomarkers. Additionally, our study population was limited to only Caucasian and African-American participants. The results of this study are preliminary and a larger study is needed to assess the performance of these biomarkers. Despite the small study, our results are consistent and encouraging. These biomarkers may have significant specificity and sensitivity to assist management in patients with cirrhosis.

In conclusion, this pilot study suggests that normal FF (≈ 0.20) with reduced RPF and GFR and increased renal arterial resistance were the key alterations in renal hemodynamics of decompensated stages of cirrhosis. As direct measurement of GFR, RPF and renal resistive indices would be impractical and costly in a clinical setting; identification of non-invasive biomarkers more accurate than creatinine which are predictive of these hemodynamic changes may lead to modifications in treatment by identifying cirrhotics at risk for HRS at a stage more amenable to therapeutic intervention. Ultimately, such early identification could perhaps decrease the need for simultaneous kidney transplantation in cirrhotics awaiting liver transplantation, and reduce mortality from kidney failure in cirrhosis.

ACKNOWLEDGEMENTS

The authors thank Jean-Pierre Raufman, M.D. (Professor of Medicine, Department of Medicine, Division of Gastroenterology and Hepatology, University of Maryland School of Medicine) for editing our manuscript and for his valuable input; Heather L. Rebeck, MT (ASCP), CLS (NCA) and Sharon Y. Huang, MT for analysis of blood samples, Reem M. Sharaf, B.A. (study coordinator), Oscar R. Del Barco, MS, RDMS, RVT, RT (R) (sonographer) and University of Maryland General Clinical Research Center Staff.

FUNDING

“The project described was supported by Grant Number 5 K23 DK089008-04 from the National Institutes of Health (NIH) National Institute of Diabetes and Digestive and Kidney Diseases (to Ayse L. Mindikoglu, M.D., M.P.H.) and its contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Institute of Diabetes and Digestive and Kidney Diseases or the NIH”

This work was also supported by the University of Maryland Clinical Translational Science Institute and the University of Maryland General Clinical Research Center.

REFERENCES

1. Garcia-Tsao G, Parikh CR, Viola A. Acute kidney injury in cirrhosis. *Hepatology*. 2008; 48:2064–77. [PubMed: 19003880]
2. Gines P. Pharmacological management of hepatorenal syndrome: lessons from non-responders. *J Hepatol*. 2011; 55:268–9. [PubMed: 21349296]
3. Gluud LL, Christensen K, Christensen E, Krag A. Systematic review of randomized trials on vasoconstrictor drugs for hepatorenal syndrome. *Hepatology*. 2010; 51:576–84. [PubMed: 19885875]
4. Cholongitas E, Senzolo M, Patch D, Shaw S, O’Beirne J, Burroughs AK. Cirrhotics admitted to intensive care unit: the impact of acute renal failure on mortality. *Eur J Gastroenterol Hepatol*. 2009; 21:744–50. [PubMed: 20160527]
5. Wong F, Nadim MK, Kellum JA, et al. Working Party proposal for a revised classification system of renal dysfunction in patients with cirrhosis. *Gut*. 2011; 60:702–9. [PubMed: 21325171]
6. Alessandria C, Ozdogan O, Guevara M, et al. MELD score and clinical type predict prognosis in hepatorenal syndrome: relevance to liver transplantation. *Hepatology*. 2005; 41:1282–9. [PubMed: 15834937]
7. Epstein M. Hepatorenal syndrome: emerging perspectives of pathophysiology and therapy. *J Am Soc Nephrol*. 1994; 4:1735–53. [PubMed: 8068872]
8. Epstein M, Berk DP, Hollenberg NK, et al. Renal failure in the patient with cirrhosis. The role of active vasoconstriction. *Am J Med*. 1970; 49:175–85. [PubMed: 5452940]
9. Epstein M, Schneider N, Befeler B. Relationship of systemic and intrarenal hemodynamics in cirrhosis. *J Lab Clin Med*. 1977; 89:1175–87. [PubMed: 864301]
10. [February 28, 2014] Data. Available at <http://optn.transplant.hrsa.gov/latestData/rptData.asp>.
11. Mindikoglu AL, Weir MR. Current concepts in the diagnosis and classification of renal dysfunction in cirrhosis. *Am J Nephrol*. 2013; 38:345–54. [PubMed: 24107793]
12. Sherman DS, Fish DN, Teitelbaum I. Assessing renal function in cirrhotic patients: problems and pitfalls. *Am J Kidney Dis*. 2003; 41:269–78. [PubMed: 12552488]
13. Kew MC, Brunt PW, Varma RR, Hourigan KJ, Williams HS, Sherlock S. Renal and intrarenal blood-flow in cirrhosis of the liver. *Lancet*. 1971; 2:504–10. [PubMed: 4105661]
14. Salerno F, Gerbes A, Gines P, Wong F, Arroyo V. Diagnosis, prevention and treatment of hepatorenal syndrome in cirrhosis. *Gut*. 2007; 56:1310–8. [PubMed: 17389705]
15. Wang DJ, Dowling TC, Meadows D, et al. Nesiritide does not improve renal function in patients with chronic heart failure and worsening serum creatinine. *Circulation*. 2004; 110:1620–5. [PubMed: 15337695]
16. Levey AS, Coresh J, Greene T, et al. Expressing the Modification of Diet in Renal Disease Study equation for estimating glomerular filtration rate with standardized serum creatinine values. *Clinical Chemistry*. 2007; 53:766–72. [PubMed: 17332152]
17. Dowling TC, Frye RF, Zemaitis MA. Simultaneous determination of p-aminohippuric acid, acetyl-p-aminohippuric acid and iothalamate in human plasma and urine by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl*. 1998; 716:305–13. [PubMed: 9824245]
18. Mindikoglu AL, Dowling TC, Weir MR, Seliger SL, Christenson RH, Magder LS. Performance of chronic kidney disease epidemiology collaboration creatinine-cystatin C equation for estimating kidney function in cirrhosis. *Hepatology*. 2014; 59:1532–42. [PubMed: 23744636]
19. Gibaldi, M.; Perrier, D. *Pharmacokinetics*. 2nd ed.. Marcel Dekker Inc; New York, NY: 1982.

20. Viazzi F, Leoncini G, Derchi LE, Pontremoli R. Ultrasound Doppler renal resistive index: a useful tool for the management of the hypertensive patient. *J Hypertens*. 2014; 32:149–53. [PubMed: 24172238]
21. SDMA-ELISA. [September 21, 2009] Available at <http://www.dld-diagnostika.de/uploads/SDMA-E.pdf>.
22. ADMA- Arginine- Plasma ELISA. [March 27, 2012] Available <http://www.dld-diagnostika.de/uploads/ADMA-Arginine-Plasma-E.pdf>.
23. Renin Human ELISA. [January 8, 2014] Available at <http://www.biovendor.com/product/immunoassays/renin-human-elisa>.
24. Aldosterone ELISA. [January 8, 2014] Available at <http://www.biovendor.com/product/immunoassays/aldosterone-elisa>.
25. Package Insert/Instructions for Use, CYSC, REF K7040, Issue date 2011-03, SIEMENS, Dimension Vista® System Flex® reagent cartridge. Siemens Healthcare Diagnostics Inc; Newark, DE:
26. Package Insert/Instructions for Use, ECREA, REF K1270A, Issue date 2012-04-04, SIEMENS, Dimension Vista® System Flex® reagent cartridge. Siemens Healthcare Diagnostics Inc; Newark, DE:
27. Package Insert/Instructions for Use, B2MIC, REF K7024, Issue date 2009-05, SIEMENS, Dimension Vista® System Flex® reagent cartridge. Siemens Healthcare Diagnostics Inc; Newark, DE:
28. Package Insert/Instructions for Use, N Latex β TP. Siemens Healthcare Diagnostics Inc; Newark, DE:
29. Quantikine® ELISA. [January 8, 2014] Human Lipocalin-2/NGAL Immunoassay. Available at <http://www.rndsystems.com/pdf/DLCN20.pdf>.
30. [January 8, 2014] Human KIM-1 Elisa Kit. Available at http://www.aviscerabioscience.com/Product_Systems/KIM-1/SK00186-01_avbs.pdf.
31. SAS software. <http://www.Sas.Com/>. The data analysis for this paper was generated using SAS software, Version 9.2 of the SAS System for Windows. Copyright © 2002-2008 SAS Institute Inc. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, NC, USA.
32. Minitab 15 Statistical Software. [Computer software]. Minitab, Inc.; State College, PA: 2007. (www.minitab.com)
33. Belcher JM, Sanyal AJ, Peixoto AJ, et al. Kidney biomarkers and differential diagnosis of patients with cirrhosis and acute kidney injury. *Hepatology*. 2013
34. [January 15, 2014] The History of Poiseuille's Law. Available at <http://www.annualreviews.org/doi/pdf/10.1146/annurev.fl.25.010193.000245>.
35. Tublin ME, Tessler FN, Murphy ME. Correlation between renal vascular resistance, pulse pressure, and the resistive index in isolated perfused rabbit kidneys. *Radiology*. 1999; 213:258–64. [PubMed: 10540669]
36. Leslie SH, Johnston B, Ralli EP. Renal function as a factor in fluid retention in patients with cirrhosis of the liver. *J Clin Invest*. 1951; 30:1200–7. [PubMed: 14888697]
37. Koeppen, BM.; Stanton, BA. Glomerular Filtration and Renal Blood Flow.. In: Koeppen, BM.; Stanton, BA., editors. *Renal Physiology*. 4th ed.. Mosby; Philadelphia: 2007. p. 31-46.
38. Hall, JE. Urine Formation by the Kidneys.. In: Hall, JEGA., editor. *Guyton and Hall textbook of medical physiology*. 12th ed.. Saunders/Elsevier; Philadelphia, PA: 2011. p. 303-322.
39. Bossard G, Bourgoin P, Corbeau JJ, Huntzinger J, Beydon L. Early detection of postoperative acute kidney injury by Doppler renal resistive index in cardiac surgery with cardiopulmonary bypass. *British journal of anaesthesia*. 2011; 107:891–8. [PubMed: 21940396]
40. Knapp R, Plotzeder A, Frauscher F, et al. Variability of Doppler parameters in the healthy kidney: an anatomic-physiologic correlation. *J Ultrasound Med*. 1995; 14:427–9. [PubMed: 7658509]
41. Rivolta R, Maggi A, Cazzaniga M, et al. Reduction of renal cortical blood flow assessed by Doppler in cirrhotic patients with refractory ascites. *Hepatology*. 1998; 28:1235–40. [PubMed: 9794906]

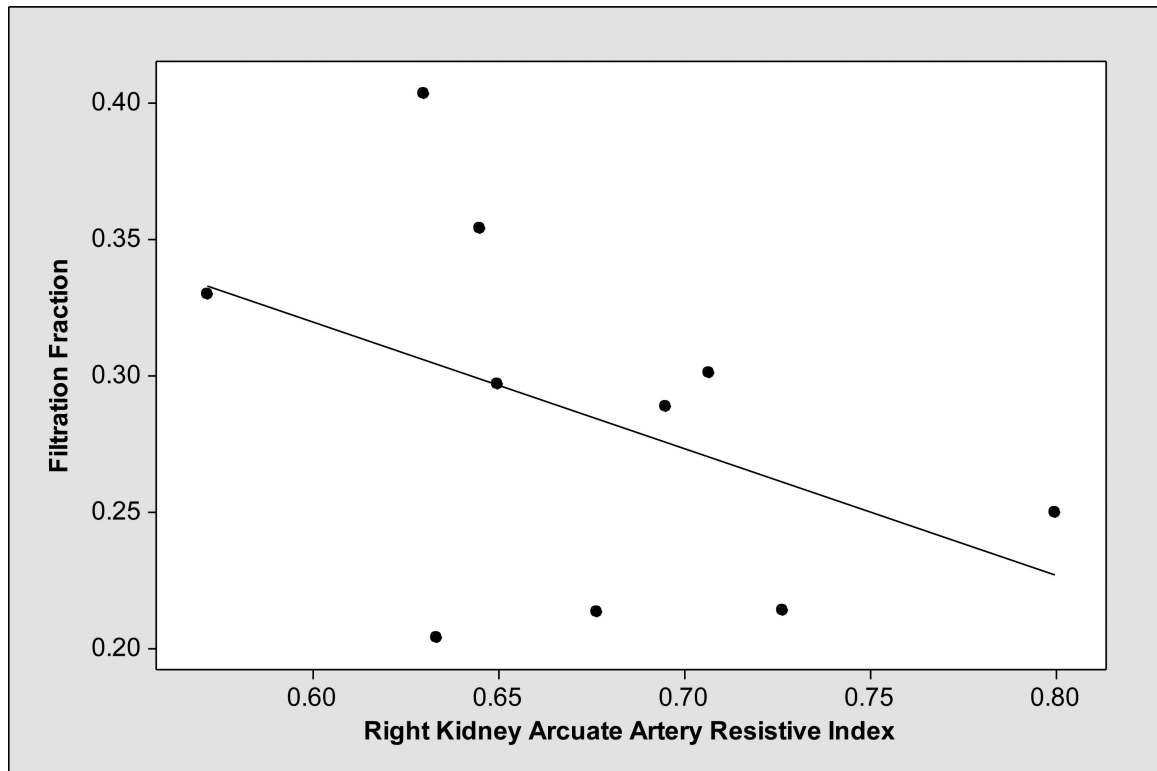


Figure 1. Right kidney arcuate artery resistive indices had medium negative correlation with filtration fraction (Pearson correlation coefficient $r = -0.44$, $P = 0.20$).

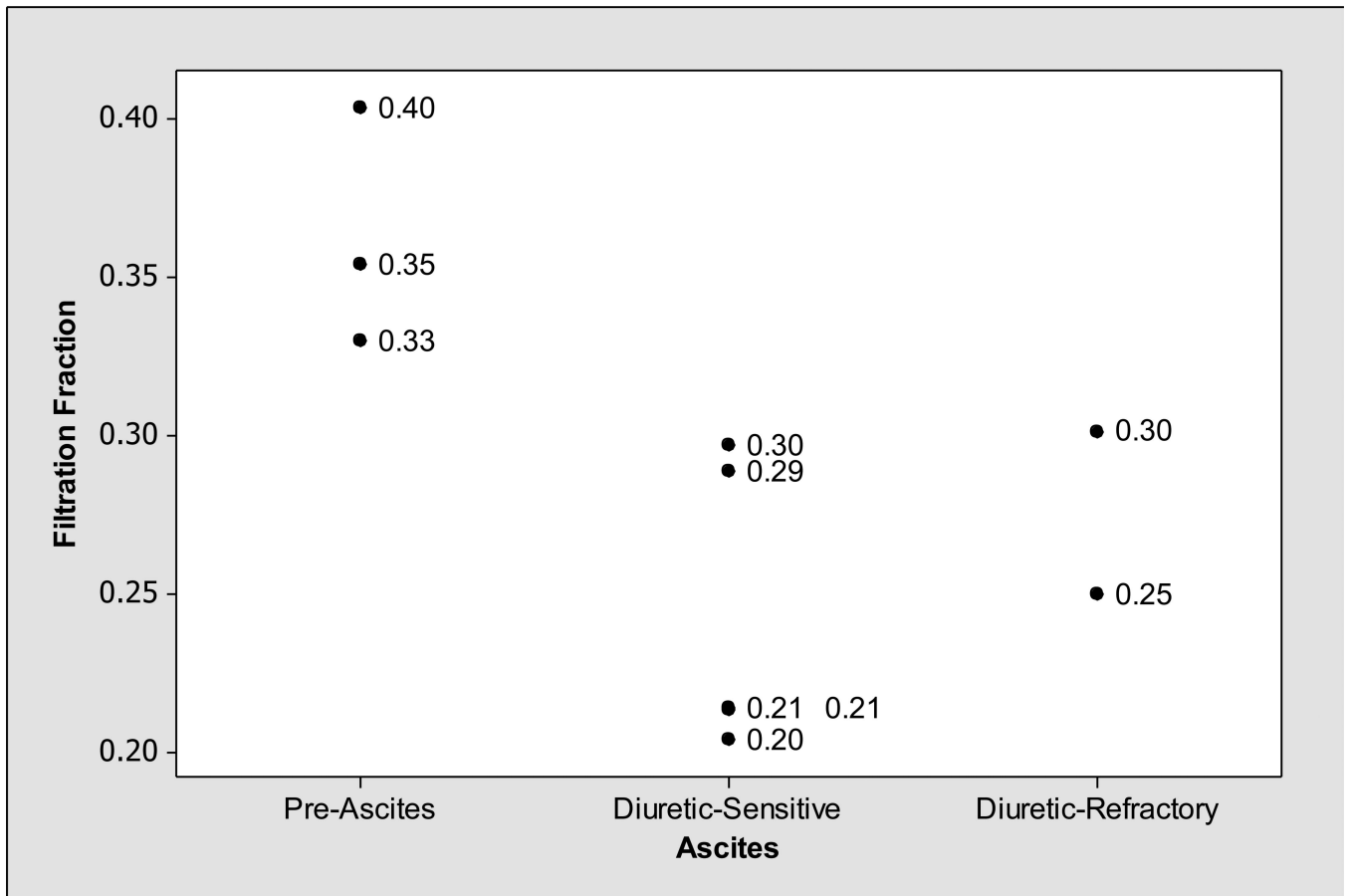


Figure 2. Filtration fraction (FF=GFR/RPF) was increased (FF>0.20) among subjects without ascites while it was lower among those with diuretic sensitive and -refractory ascites compared to those without ascites.

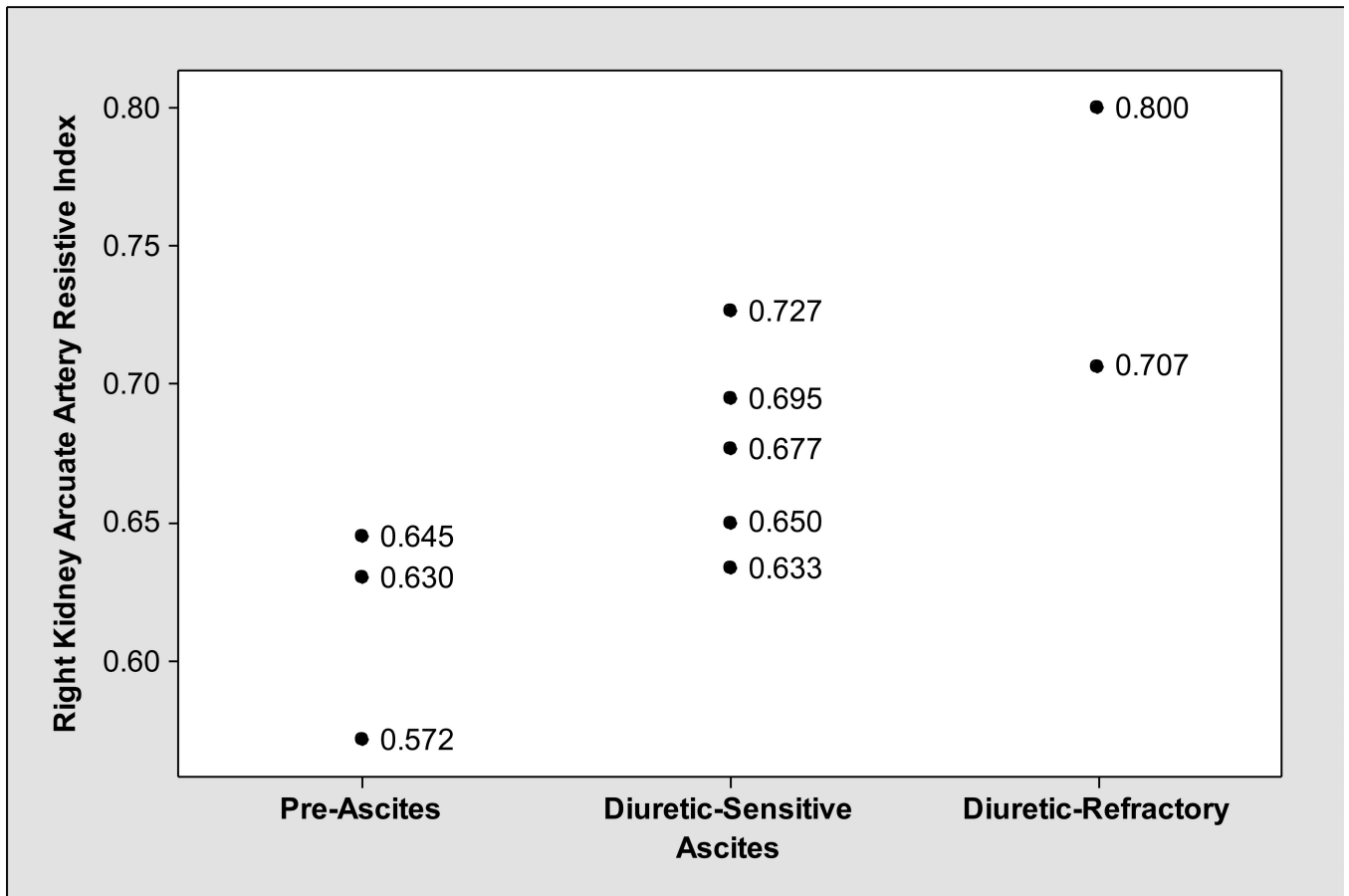


Figure 3.

In contrast to FF that was lower in subjects with ascites (Figure 2), right kidney arcuate artery (located at the junction of renal cortex and medulla; supplying renal cortex) resistive indices were elevated in those with ascites compared to those without ascites.

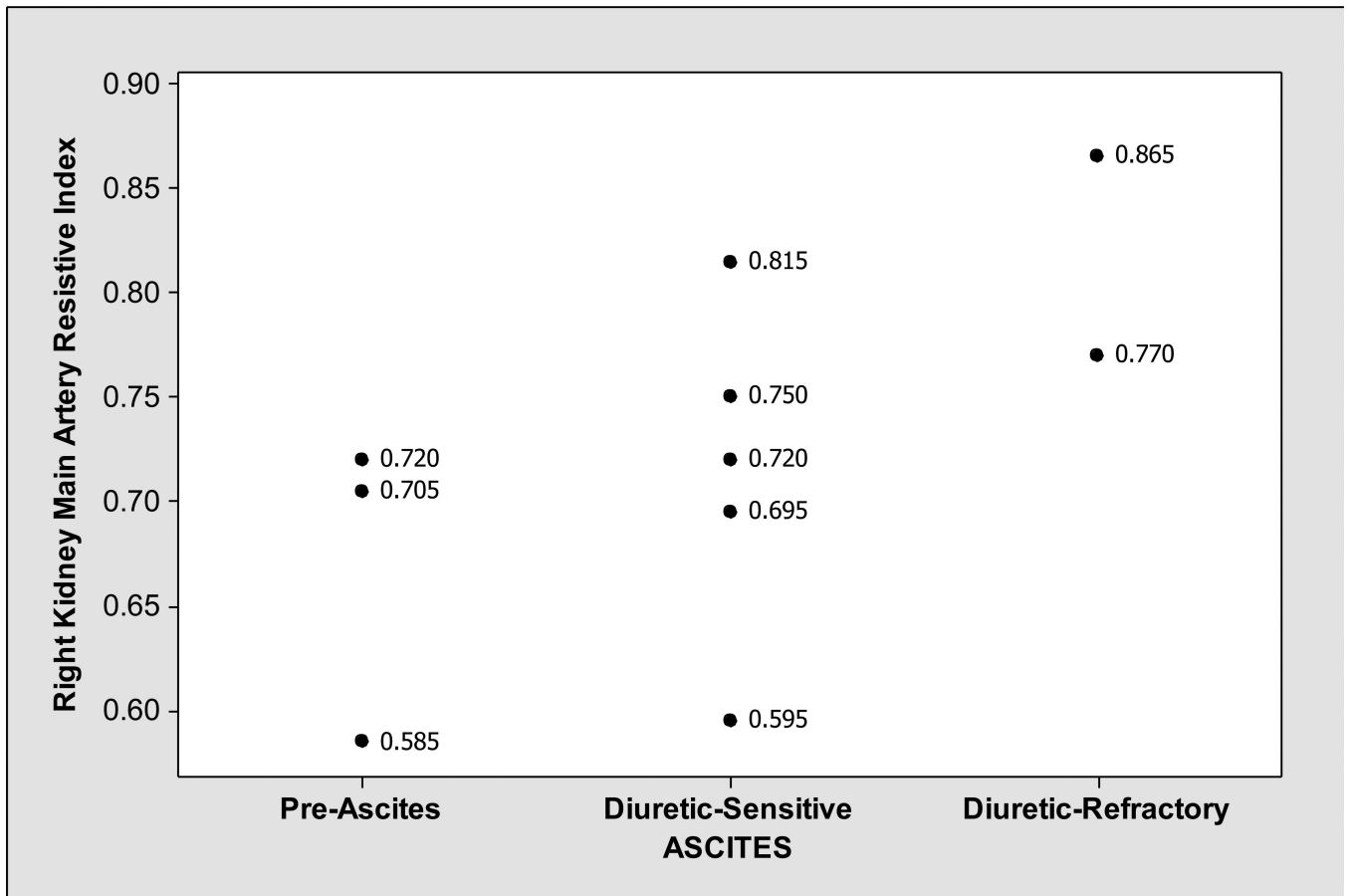


Figure 4.

In contrast to FF that was lower in subjects with ascites (Figure 2), right kidney main artery resistive indices were elevated in those with ascites compared to those without ascites.

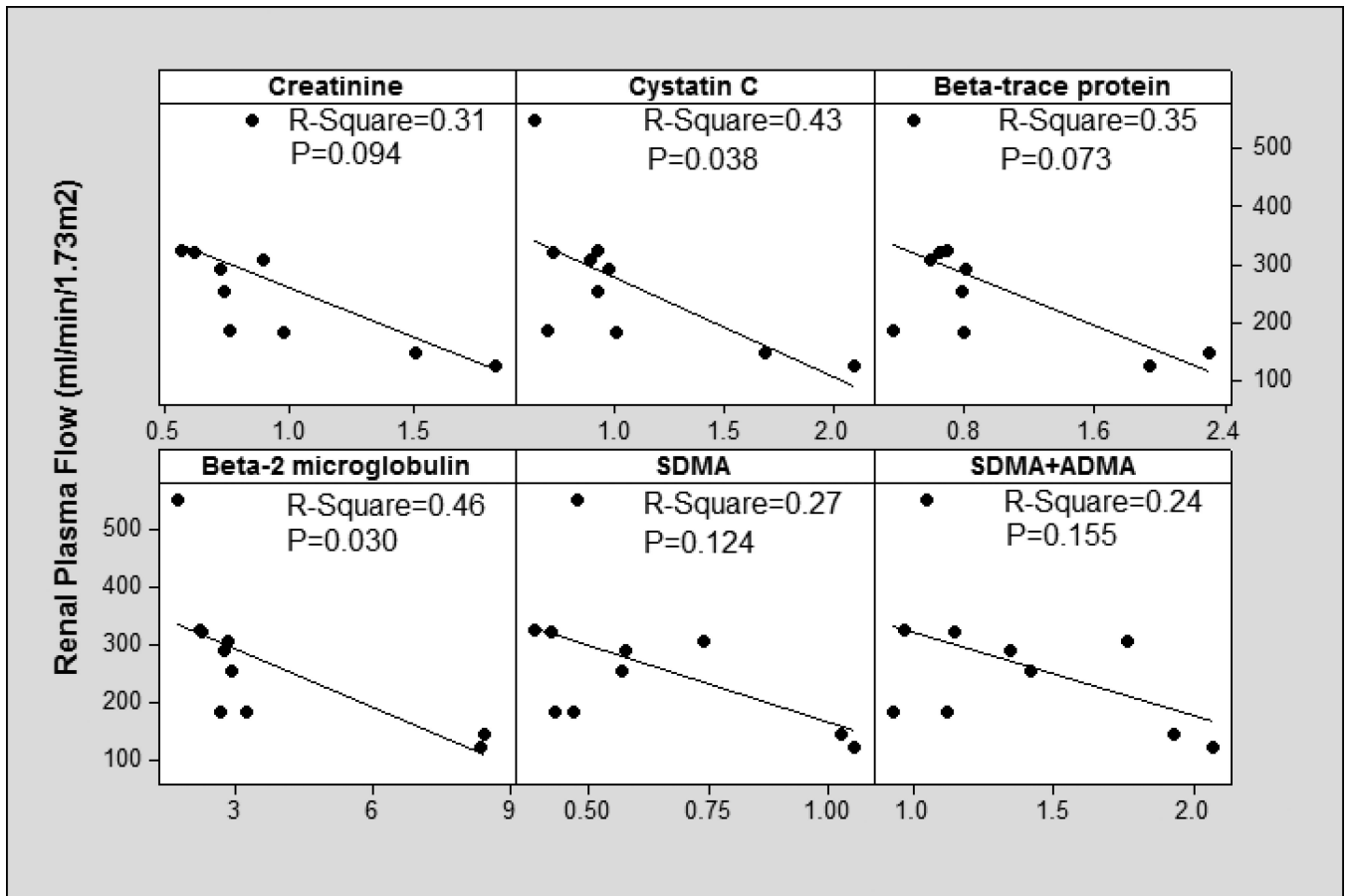


Figure 5.
 We measured candidate biomarkers simultaneously with RPF (adjusted for body surface area) and compared their performances to serum Cr.

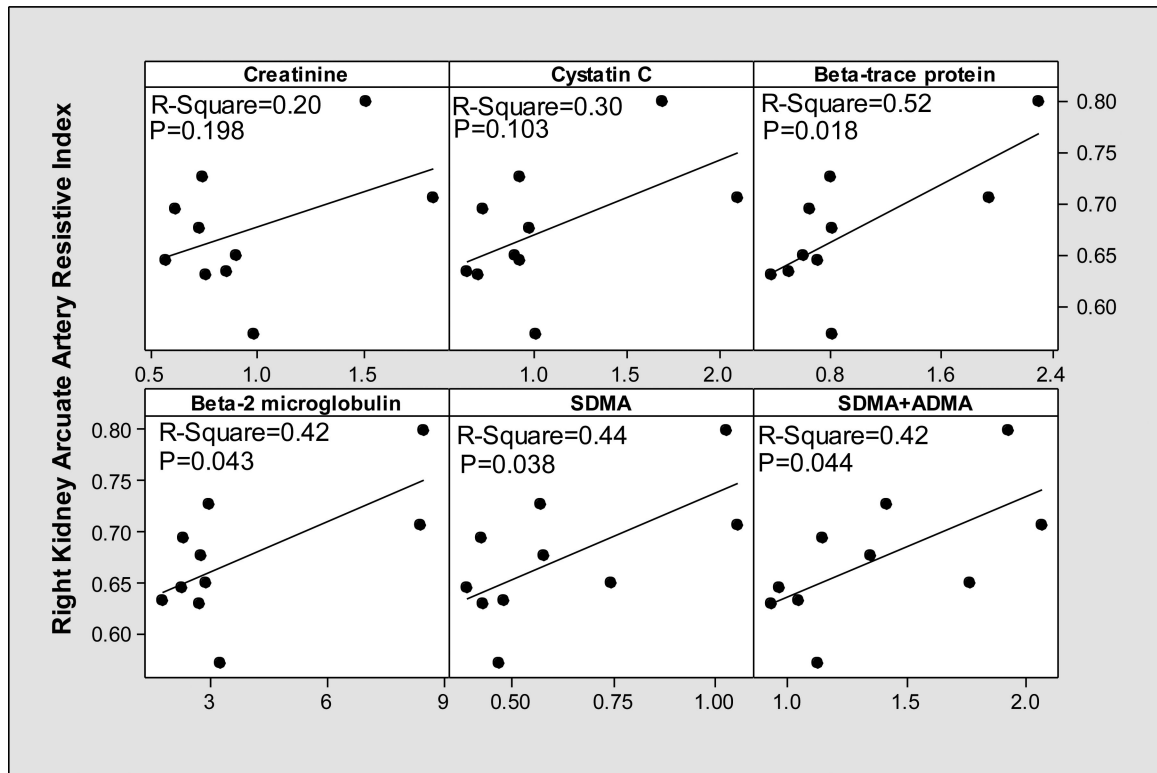


Figure 6. We measured candidate biomarkers simultaneously with right kidney arcuate artery resistive indices and compared their performances to serum Cr.

Trace Protein (mg/L)	Beta-2 Microglobulin (mg/L)	SDMA (micromol/L)	ADMA (micromole/L)	L-Arginine (micromole/L)	Renin (pg/ml)	Aldosterone (pg/ml)	NGAL (ng/ml)	Urine KIM-1 (pg/ml)	Spot Urine Protein/Cr Ratio
2.300	8.480	1.027	0.901	78.554	170.620	1638.95	2.99	851.13	0.10
0.495	1.740	0.477	0.568	87.249	76.367	1355.70	26.85	1644.32	0.05
0.791	2.940	0.568	0.848	89.733	112.210	685.38	133.40	1188.20	0.25
0.594	2.860	0.742	1.022	81.882	35.130	347.29	9.60	1972.18	0.09
0.367	2.690	0.427	0.501	67.139	5.370	191.97	5.27	390.50	0.11
1.940	8.400	1.055	1.013	97.693	29.584	333.03	88.21	6992.23	0.17
0.697	2.230	0.387	0.576	75.672	29.896	187.57	4.83	5520.12	0.08
0.805	2.750	0.575	0.768	160.220	149.770	1115.60	19.85	848.62	0.09
0.648	2.280	0.422	0.723	133.200	1.307	232.45	5.23	2400.70	0.04
0.803	3.250	0.467	0.655	73.798	1.168	205.31	13.08	99.44	0.06

Table 2

Simultaneous Measurement of Renal Hemodynamics including GFR, RPF, and Renal Resistive Indices in Patients with Cirrhosis

Case #	Type of Ascites	IVC Diameter (cm)	GFR ¹	RPF ¹	FF ²	Right Kidney Main Artery Resistive Index ³	Right Kidney Segmental Artery Resistive Index ³	Right Kidney Interlobar Artery Resistive Index ³	Right Kidney Arcuate Artery Resistive Index ³	Left Kidney Main Artery Resistive Index ³	Left Kidney Segmental Artery Resistive Index ³	Left Kidney Interlobar Artery Resistive Index ³	Left Kidney Arcuate Artery Resistive Index ³
1	Diuretic-Refractory	1.367	36.193	144.950	0.25	0.865	0.773	0.740	0.800	0.805	0.763	0.768	0.635
2	Diuretic-Sensitive	1.970	112.216	550.298	0.20	0.595	0.672	0.643	0.633	0.670	0.612	0.605	0.592
3	Diuretic-Sensitive	1.550	54.217	253.319	0.21	0.750	0.727	0.680	0.727	0.830	0.795	0.803	0.708
4	Diuretic-Sensitive	1.630	91.011	306.801	0.30	0.695	0.677	0.663	0.650	0.725	0.677	0.668	0.622
5	No Ascites	1.550	73.901	183.233	0.40	0.720	0.665	0.687	0.630	0.680	0.683	0.650	0.673
6	Diuretic-Refractory	2.063	36.819	122.263	0.30	0.770	0.748	0.728	0.707	0.775	0.762	0.750	0.763
7	No Ascites	2.190	114.575	323.786	0.35	0.705	0.665	0.648	0.645	0.765	0.627	0.683	0.662
8	Diuretic-Sensitive	1.108	61.775	289.812	0.21	0.815	0.743	0.718	0.677	0.795	0.732	0.683	0.712
9	Diuretic-Sensitive	1.607	92.458	320.475	0.29	0.720	0.712	0.693	0.695	0.745	0.697	0.712	0.658
10	No Ascites	1.070	60.205	182.552	0.33	0.585	0.593	0.572	0.572	0.585	0.630	0.552	0.585

¹ GFR and RPF were reported in ml/min/1.73m².

² Filtration Fraction (FF)=GFR/RPF.

³ Two repeated measurements were taken from each renal artery; renal resistive index of each artery was reported as the mean value of these two measurements. Final resistive index was reported as the mean value of the mean resistive indices of the upper, mid and lower segmental, interlobar and arcuate arteries.

Table 3 Mean GFR, RPF, FF and Renal Resistive Indices Stratified by Type of Ascites in Patients with Cirrhosis

	Right Kidney	Mean Renal Resistive Index	Left Kidney	Mean Renal Resistive Index	Mean GFR ¹	Mean RPF ¹	Mean FF ²
No Ascites	Main Renal Artery	0.670	Main Renal Artery	0.677	82.894	229.857	0.36
	Segmental Artery	0.641	Segmental Artery	0.647			
	Interlobar Artery	0.636	Interlobar Artery	0.628			
	Arcuate Artery	0.616	Arcuate Artery	0.640			
Diuretic-Sensitive Ascites	Main Renal Artery	0.715	Main Renal Artery	0.753	82.336	344.141	0.24
	Segmental Artery	0.706	Segmental Artery	0.702			
	Interlobar Artery	0.680	Interlobar Artery	0.694			
	Arcuate Artery	0.676	Arcuate Artery	0.658			
Diuretic-Refractory Ascites	Main Renal Artery	0.818	Main Renal Artery	0.790	36.506	133.606	0.28
	Segmental Artery	0.761	Segmental Artery	0.763			
	Interlobar Artery	0.734	Interlobar Artery	0.759			
	Arcuate Artery	0.753	Arcuate Artery	0.699			

¹ GFR and RPF were reported in ml/min/1.73m².

² FF=GFR/RPF.