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Urinary and systemic angiotensinogen in Heart Failure patients

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Abstract

Introduction

Heart failure (HF) is a common chronic disabling disease responsible for high levels of morbidity and mortality and marked economic burden. Chronic renin-angiotensin system (RAS) activation leads to long-term deleterious effects in HF. Angiotensin-converting enzyme inhibitors (ACEi) and angiotensin II receptor blockers (ARB) are able to modulate the RAS. Urinary angiotensinogen (AGT) was recently considered a biomarker of local intrarenal RAS activity. We aimed at characterizing plasma and urinary AGT concentration (1) in mild (NYHA I-II) and severe (NYHA III-IV) CHF patients and (2) in CHF patients treated with either ACEi or ARBs.

Material and methods

Sixty patients with stable CHF were selected. Data on NYHA functional class and chronic medication were taken. Blood and urine samples were drawn. AGT concentrations were determined using a commercial ELISA kit.

Results

When compared to mild CHF patients, severe patients had higher aldosterone concentrations and ACE activity, and those without chronic renal failure had higher urinary AGT excretion. ARB-only treated patients had higher plasma AGT

concentrations and ACE activity than ACEi-only treated patients. However, no difference was found in urinary AGT.

Conclusions

Severe CHF activates systemic RAS and renal RAS in the presence of non-failing kidneys. ARBs might increase plasma AGT concentration.

Keywords: Angiotensinogen, heart failure, angiotensin II receptor blockers, angiotensin converting enzyme inhibitors, renin-angiotensin system.

Introduction

Heart failure (HF) is a common chronic disabling disease responsible for high levels of morbidity and mortality as well as marked economic burden[1]. Most commonly related to the elderly, its incidence and prevalence are both increasing due to populational ageing and also to improved medical care[2]. In Portugal, its estimated overall prevalence lies around 4.36% in over 25 years old adults, according to the EPICA study[3]. It affects both genders equally[3]. The *New York Heart Association* (NYHA) suggests a clinical division in four stages (I-IV) of severity of disease, taking into account patients daily activity functional limitations due to the disease. It is used to appraise the status of patients with heart disease and evaluate treatment outcomes in clinical and research settings[4,5]. This division correlates with both morbidity and mortality in HF[6,7].

Although HF has more than a few etiologies, they all have in common a chronic decrease in cardiac output (CO) and low systemic perfusion[2]. These hemodynamic changes lead up to sustained activation of multiple neuro-hormonal systems, like the sympathetic nervous system and the renin-angiotensin system (RAS)[2,8]. Chronic RAS activation contributes to long-term deleterious effects in HF, like renal sodium reabsorption, increased bradykinin hydrolysis, vascular remodelling, myocardial hypertrophy and fibrosis[9]. For that, one of the cornerstones of chronic HF (CHF) treatment is the RAS blocking effect of angiotensin-converting enzyme inhibitors (ACEi) and angiotensin II AT_1 receptor blockers (ARBs)[10]. The "classic" RAS has long

been studied. Systemic angiotensinogen (AGT) is mainly synthesized and released into the circulation by the liver's hepatocytes, although there is also a contribution of local RAS systems[11]. AGT is cleaved by renin, mainly synthesized by the juxtaglomerular apparatus, to angiotensin I, which is further cleaved by angiotensin converting enzyme (ACE) to angiotensin II (AngII), the main effector of the "classical" RAS. The synthesis of AGT relies for the most part on chronic regulatory mechanisms modulated by estrogen, interleukins, cortisol, thyroid hormones and AngII[11]. This regulation is especially important as AGT plasma concentration, together with renin, are the rate limiting step in angiotensin I generation. The synthesis of renin by the juxtaglomerular apparatus is inhibited by AngII, functioning as a negative feedback loop[12]. Furthermore, Ang II is able to increase AGT mRNA stability through a decrease in cAMP believed to be mediated by AngII AT_1 receptors[13]. Until now, only one study[14] has measured angiotensin I, AngII, plasma renin activity (PRA) and aldosterone in CHF patients. However, this study was limited to severe patients receiving therapy with ACEi.

Recently, the RAS has been expanded and the focus turned to local RAS, namely the intrarenal RAS[15,16]. These local systems are said to be activated at an earlier stage of HF than the systemic RAS[2]. In fact, AngII renal levels have been shown to be markedly higher than plasma AngII levels due to both local generation of AngII and accumulation of plasma AngII mediated by AngII AT_1 receptors[17,18]. Since plasma AGT is unable to pass through a healthy glomerular filtration barrier, all the renal AGT must be formed

locally[19]. In fact, both AGT and renin mRNA have been shown to be present in renal proximal tubule cells[20] and ACE protein has been identified on the apical membrane of the proximal tubule[21]. Thus, the RAS cascade can occur in the tubular lumen. Chronic upregulation of local RAS has been shown to produce renal cell lesion, fibroblast proliferation and fibrosis[22], as well as increased sodium and water retention in the distal tubule[23,24]. Circulating and renal RAS are associated since plasma AngII increases renal proximal tubule AGT production, as does in the liver[13,25]. However, studies failed to show a direct correlation between plasma AngII levels and renal AGT concentrations. Urinary AGT has been shown to be a marker of renal RAS status, namely in IgA nephropathy[26], childhood type 1 diabetic nephropathy[27], chronic kidney disease[28,29] and hypertension[30]. However, a recent paper questions whether urinary renin is a better biomarker of renal RAS than urinary AGT[31]. Concerning CHF, there are still no reports on plasma AGT concentration or urinary AGT excretion along with disease severity.

Even though CHF treatment relies on ACEi and ARBs, RAS blocking drugs can modulate the plasma concentration of the RAS components, eventually counteracting their own-mediated initial RAS inhibition. Thus, ACEi and ARBs are said to increase renin, whether concentration or activity, and angiotensin I concentration when compared to non-treated patients[32]. Plus, ARBs are expected to increase AngII plasma concentration while ACEi have an opposite effect and decrease AngII formation[32]. Although, ACEi escape was estimated to occur in up to 45% of severe CHF patients

(NYHA classes III and IV)[14]. RAS modulation is gaining increasing importance as the knowledge on alternative RAS pathways increases, namely that on the renin/(pro)renin receptor which might have negative effects in renal and heart failure, partially offsetting currently-used RAS blockers benefits[32]. Thus, in the present study, we aimed at characterizing (1) plasma and renal RAS in patients with mild and severe CHF, and (2) plasma and renal RAS in patients treated with RAS blocking drugs (either ACEI or ARBs).

Materials and methods

The project was approved by the Health Ethics Commission of the Hospital S. João (HSJ). Sixty one patients with CHF were selected from the Heart Failure Clinic of HSJ, informed about the study and asked to participate giving their informed consent. On the day of the visit, a brief physical exam was performed, left arm systolic (SBP) and diastolic (DBP) blood pressure were measured using an automated blood pressure monitor, and functional status (NYHA classes I-IV) was evaluated. Data on age, weight, gender and chronic medication were taken and blood and spot urine samples collected. Plasma and urinary AGT concentrations were determined using a commercial ELISA kit (IBL#27412). Plasma renin concentration, ACE activity, aldosterone, brain natriuretic peptide (BNP), serum creatinine, spot creatinine and cystatin C (CysC) concentrations were quantified using commercial kits and an automated biochemical analyzer. Glomerular filtration rate (GFR) was estimated using the Cockcroft-Gault

equation. Urinary AGT was normalized for creatinine excretion. Sample data was normalized for gender when appropriated. Statistical analysis was performed using independent samples t-test, Man-Whitney or Spearman correlation coefficient, as appropriate. A significance level of 5% was considered.

Results

Patients were first divided in two groups taking into account their NYHA functional status: mild for NYHA classes I and II or severe for NYHA classes III and IV. Severe CHF patients were significantly older and weighted less than mild CHF patients (table 1). SBP and DBP were similar between mild and severe CHF patients (table 1). Since we found a difference in gender distribution between these two groups (table 1), all other data related to this part of the study was normalized for gender. Severe CHF patients showed significantly higher plasma BNP and CysC concentrations as well as lower GFR than mild CHF patients (figure 1). Severe CHF patients had similar plasma AGT and renin concentrations but higher ACE activity and aldosterone concentration than milder CHF patients (figure 2).

In a second analysis, patients were divided into four groups according to their use of RAS blocking drugs (ACEi-only, ARB-only, both ACEi and ARB or no RAS blocker). Only ACEi-only and ARB-only were compared because the number of patients on ACEi plus ARB or with no RAS blocker drug was very small. There were no significant differences in age, weight or gender between treatment groups (table 2).

BNP, CysC and GFR were similar between patients from the two treatment groups (figure 3). ARB-only treated patients had significantly higher plasma AGT concentration and ACE activity than ACEi-only patients, although there were no significant differences in renin or aldosterone concentration (figure 4).

There were no differences in urinary AGT excretion, neither between mild and severe CHF patients, nor between ARB-only and ACEi-only treatment groups (figure 5). The decrease in renal function is one of the strongest predictors of mortality in advanced HF[25] and obviously influence data taken out from urine samples. So, in order to better analyze the results concerning the urinary excretion of AGT, patients were divided in two kidney disease groups according to their GFR estimation: those with or without chronic renal failure (CRF), setting the value of 60 mL/min/1.73m^2 as the cut-off point as stated in the National Kidney Foundation guidelines for chronic kidney disease[33]. CHF patients with CRF had significantly higher urinary AGT excretion than patients without CRF (figure 6A). When we looked for differences between CHF severity, we found that there was no difference in urinary AGT excretion between mild and severe CHF patients with CRF (figure 6B) but that, in the absence of CRF, urinary AGT excretion was significantly higher in severe than in mild CHF patients (figure 6C). These severe CHF patients without CRF had significantly higher BNP but not Cystatin C or GFR than milder CHF patients (figure 7). Moreover, aldosterone concentrations and ACE activity were also higher in severe than in milder CHF patients without CRF

(figure 8). In these patients, there was a significant positive correlation between urinary AGT excretion and DBP (r^2=0.802; p=0.01) but not with SBP (r^2=0.204; p=0.73) or plasma AGT concentration (r^2=0.509; p=0.31) (figure 9).

Discussion

The main findings of the present work were that severe CHF patients show increased systemic RAS activity and that, those with preserved renal function, also show increased renal RAS activation when compared with milder CHF patients. Moreover, data suggest that ARB treatment is associated with increased plasma AGT concentration when compared to ACEi treatment in CHF patients.

Grouping patients using only a clinical parameter (NYHA class) might seem reductive and simplistic. Nonetheless, NYHA class stratification is an easily accessible clinical instrument to appraise CHF severity[34] and correlates with both disease morbidity and mortality[6]. Accordingly, we measured BNP, CysC and Creatinine, as biomarkers of heart and renal function, to further validate our clinical assessment. With all due limitations, an even more reductive approach was followed, as a division in two groups was performed since our sample was relatively small in order to accomplish four considerably sized groups. As expected, severe CHF patients had significantly higher concentrations of BNP and CysC, as well as lower estimated GFR, confirming that the severe group had, in fact, a worst functional status than milder CHF patients. The normal epidemiological distribution of CHF patients along NYHA functional class

resembles that of a pyramid, being NYHA class IV only a small percentage of overall CHF pool of patients[3,35], which probably determined the different size of disease severity groups (n=35 vs 25). Unexpectedly, there was also a discrepancy in gender, as there were fewer females in the mild CHF group. Since the reason for the discrepancy was most probably an unplanned selection bias rather than an actual decrease in mild female patients in the Heart Failure Clinic of HSJ, our statistical analysis had to be normalized for gender.

HF is a chronic progressive disease in which state-of-the-art medical treatment aims at not curing, but managing and delaying its rate of progression. Hence, severe CHF patients were older than milder CHF patients. Cardiac cachexia has long been associated with advanced stages of CHF[36]. Plus, it is mediated by pro-inflammatory cytokines, namely TNF-α, and is independent from poor nutrition[37]. In this study, severe CHF patients weighted less than milder CHF patients, which is probably explained by the higher prevalence of cardiac cachexia in severe CHF patients.

Severe CHF patients had higher plasma ACE activity than milder CHF patients, which possibly favors higher AngII concentrations in severe CHF patients. An increased concentration of AngII might also explain the increased aldosterone concentration found in severe CHF patients, as AngII is able to increase both aldosterone synthesis and secretion[38,39]. Even if renal injury and the consequent alterations in potassium equilibrium could play a role in this modulation, severe patients without CRF also had a

higher ACE activity and aldosterone concentrations then milder CHF patients. Thus, we believe that AngII might be increased in severe CHF patients, leading to the perpetuation of long-term deleterious effects of chronic RAS activation.

The effects of RAS blocking drugs on RAS peptide levels are gaining increasing interest. Patients were initially divided into four groups, namely ACEi-only, ARB-only, both ACEi and ARB or non-blocked CHF patients. However, the two latter groups were rather small, and so we did not include them in the statistical analysis. ACEi are a first-line treatment in CHF with left ventricle ejection fraction (LVEF) <40%[10]. ARBs are only recommended in CHF patients with LVEF <40% which are intolerant to ACEi or show persistent symptoms despite therapy with an ACEi and a β-blocker[10]. The notorious size discrepancy that we found in our sample (n=42 vs 6 for ACEi-only and ARB-only, respectively) is probably a reflection of these treatment guidelines. Globally the ACEi-only and ARB-only groups were similar in what concerns demographic parameters, including the NYHA functional class. Additionally, there were no differences between BNP, CysC or GFR, suggesting that both pharmacological approaches have the same effect on cardiac and renal dysfunction associated with CHF. As expected, ARB-only treated patients had higher plasma ACE activity as compared to ACEi-only treated patients. Nonetheless, three ACEi-only treated patients showed a deviant behavior, having far more ACE activity than the remaining patients on ACEi, which might represent poor treatment compliance or reflect ACE escape. ARB-only

treatment in CHF was also associated with an increase in plasma AGT concentration when compared to ACEi-only treatment.

AngII is able to increase both AGT and aldosterone synthesis by the liver and suprarenal, respectively[39,40]. Although AngII AT_1 receptors mediate both effects, the signal transduction pathways differ[41]. In the hepatocyte, AGT mRNA is present in a remarkable excess when compared to the AGT protein that is actually secreted and the majority of AGT is intracellulary degraded, never getting to be secreted[11]. Although AngII is the most powerful stimulator of AGT release by the liver *in vitro,* it is not the most important *in vivo*[11,42]. AngII is able to stabilize AGT mRNA avoiding its degradation in the Hep2GC cells, leading to an increased AGT release[13,43]. This is believed to happen through an AngII AT1-dependent cAMP decrease, which was shown to be inhibited by ARBs, namely losartan, *in vitro*[43]. However, no data exists concerning *in vivo* conditions. This positive feedback loop is believed to exist in order to support states of high AngII demand[44].

ARBs are selective and competitive AngII AT_1 receptor antagonists. Losartan and candesartan are the only two ARBs approved by the FDA for CHF treatment. Both have relatively small biological half-lives (6~9 and 9 hours, respectively) and are used as a single daily dose administered in the morning[10]. This might allow that in a minor, yet significant, portion of the day AngII AT_1 receptors might be free to bind AngII. The daily balance at the slower genomic level might favor an AT_1-block "phenotype", as

seen in the lack of difference in aldosterone concentration between ACEi and ARB-only treatment groups. However, at the faster post-transduction AGT mRNA stabilization induced by AngII, it may lead to a temporary increase in AGT secretion rate, which would justify the higher plasma AGT concentration found in ARB-only treated CHF patients compared to those on ACEi-only treatment. Since AGT has a significant longer half-life (an initial rapid phase of 1 hour followed by a second slower phase of 8 hours[45]), this minor "free from ARB" time may condition a global increase in daily or only morning AGT secretion but not in aldosterone concentration. In the present study blood samples were collected early in the morning and better reflect the period of ARB absence and, eventually, high AngII concentrations. Furthermore, losartan has already been show to offer a worst daily blood pressure control, as measured by daily mean ambulatory blood pressure (ABP) measurement, when compared to olmesartan, a longer acting ARB[46,47]. One study further added that this effect was clearly obvious in the last 2 and 4 hours of ABP measurements[48]. In the future, it might be important to characterize the circadian variations in AGT concentrations. Another question to be solved in the near future is that the increase in plasma AGT concentration observed in ARB-treated patients (vs ACEi-only) might favor an increase in ACE2 activity and, consequently, angiotensin (1-7) formation, which might have beneficial effects in the failing heart and kidney and, thus, play a role on the beneficial effects of ARB treatment in CHF.

The local RAS also has an important role in the pathophysiology of CHF[49]. A significant part of the consequences of chronic RAS activation are a direct result of local RAS actions[2,49,50]. As part of our work, we also aimed at characterizing the local renal RAS in CHF, as the kidney has an important role in the hypervolemic state associated with this disease[2]. Urinary AGT has been validated as a biomarker of renal RAS activity[19] in IgA nephropathy[26], childhood type 1 diabetic nephropathy[27], chronic kidney disease[28,29] and hypertension[30]. Theoretically, it would be better to determine urinary AGT in a 24h urine sample since it would provide a better perspective on the overall daily renal RAS activation. However, it would require costly enzyme inhibitors and a greater contribution from the patient himself. Therefore urinary AGT determined in spot urine sample and normalized to creatinine excretion rate stands as a valid, reliable and simpler alternative[19]. Plus, it could be translated to the clinical setting with ease. Although renal lesion and function markers, CysC and Creatinine, were significantly higher and GFR lower in severe CHF patients, there were no statistically significant differences in spot urinary AGT excretion between disease severity groups or between ACEi-only and ARB-only treatment. In order exclude the impact of CRF to our data on urinary AGT we further divided patients according to their GFR estimation, into two kidney disease groups – those with and those without CRF – using the GFR value of 60 mL/min/1.73m^2 as the cut-off point. As expected and already demonstrated in the past[28,29], CRF patients had higher levels of urinary AGT excretion than patients

without CRF, probably reflecting the filtration of plasma AGT which would normally not occur in the presence of an intact glomerular barrier. Alternatively, this may indicate that the local intrarenal RAS is already at its maximal activation by CRF and so CHF will not have a significant impact as in patients without CRF. We further subdivided patients in disease severity groups and observed that severe CHF patients without CRF (but not those with CRF) had higher urinary AGT excretion when compared to milder CHF patients, even in the presence of similar estimated GFR or CysC. This severe patients group had still higher aldosterone concentrations. Elevated AngII concentration in severe CHF patients might also be the rationale behind the increase in urinary AGT excretion in patients without CRF, since AngII stimulates the production of AGT at the proximal tubule[25]. This may portray that the high levels of AngII associated with severe CHF may additionally lead to an upregulation of the local intrarenal RAS even if there is no primary renal injury. In the future it will be important to measure plasma renin and prorenin activities, ACE2 activity and AngII concentrations in order to complete RAS characterization in CHF patients and aid enlighten our hypothesis about elevated AngII in severe and ARB-only treated CHF patients.

Conclusions

Severe CHF patients show increased activity of the systemic RAS when compared with milder CHF patients. Moreover, severe CHF patients without CRF had increased urinary AGT excretion than milder CHF patients, suggesting intrarenal RAS activation

in severe CHF patients with non-failing kidneys. Finally, treatment with an ARB might be associated with increased plasma AGT concentrations. Future experiments measuring AngII concentration in these patients will establish whether increased AngII concentration in severe CHF patients and in ARB-only treated CHF patients might explain our data.

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Declaration of conflicting interest

The author declares that there is no conflict of interest.

References

1. Liao L, Allen LA and Whellan DJ. Economic burden of heart failure in the elderly. *Pharmacoeconomics*. 2008; 26: 447-62.

2. Gomes MC, Ferreira A and Bettencourt P. [Physiopathology of heart failure]. *Rev Port Cardiol*. 2004; 23 Suppl 2: II7-23.

3. Ceia F, Fonseca C, Mota T, et al. [Epidemiology of heart failure in mainland Portugal: new data from the EPICA study]. *Rev Port Cardiol*. 2004; 23 Suppl 3: III15-22.

4. Severo M, Gaio R, Lourenco P, Alvelos M, Bettencourt P and Azevedo A. Indirect calibration between clinical observers - application to the New York Heart Association functional classification system. *BMC research notes*. 2011; 4: 276.

5. Bennett JA, Riegel B, Bittner V and Nichols J. Validity and reliability of the NYHA classes for measuring research outcomes in patients with cardiac disease. *Heart & lung : the journal of critical care*. 2002; 31: 262-70.

6. Barsheshet A, Shotan A, Cohen E, et al. Predictors of long-term (4-year) mortality in elderly and young patients with acute heart failure. *Eur J Heart Fail*. 2010; 12: 833-40.

7. Pocock SJ, Wang D, Pfeffer MA, et al. Predictors of mortality and morbidity in patients with chronic heart failure. *Eur Heart J*. 2006; 27: 65-75.

8. Seixas-Cambao M and Leite-Moreira AF. Pathophysiology of chronic heart failure. *Rev Port Cardiol*. 2009; 28: 439-71.

9. Adams KF, Jr. Pathophysiologic role of the renin-angiotensin-aldosterone and sympathetic nervous systems in heart failure. *Am J Health Syst Pharm*. 2004; 61 Suppl 2: S4-13.

10. Dickstein K, Cohen-Solal A, Filippatos G, et al. ESC guidelines for the diagnosis and treatment of acute and chronic heart failure 2008: the Task Force for the diagnosis and treatment of acute and chronic heart failure 2008 of the European Society of Cardiology. Developed in collaboration with the

Heart Failure Association of the ESC (HFA) and endorsed by the European Society of Intensive Care Medicine (ESICM). *Eur J Heart Fail*. 2008; 10: 933-89.

11. Morgan L, Broughton Pipkin F and Kalsheker N. Angiotensinogen: molecular biology, biochemistry and physiology. *The international journal of biochemistry & cell biology*. 1996; 28: 1211-22.

12. Schweda F and Kurtz A. Regulation of renin release by local and systemic factors. *Reviews of physiology, biochemistry and pharmacology*. 2012; 161: 1-44.

13. Deschepper CF. Angiotensinogen: hormonal regulation and relative importance in the generation of angiotensin II. *Kidney Int*. 1994; 46: 1561-3.

14. van de Wal RM, Plokker HW, Lok DJ, et al. Determinants of increased angiotensin II levels in severe chronic heart failure patients despite ACE inhibition. *Int J Cardiol*. 2006; 106: 367-72.

15. Zhuo JL and Li XC. New insights and perspectives on intrarenal renin-angiotensin system: focus on intracrine/intracellular angiotensin II. *Peptides*. 2011; 32: 1551-65.

16. Cat AN and Touyz RM. A new look at the renin-angiotensin system--focusing on the vascular system. *Peptides*. 2011; 32: 2141-50.

17. Campbell DJ, Lawrence AC, Towrie A, Kladis A and Valentijn AJ. Differential regulation of angiotensin peptide levels in plasma and kidney of the rat. *Hypertension*. 1991; 18: 763-73.

18. Zou LX, Imig JD, von Thun AM, Hymel A, Ono H and Navar LG. Receptor-mediated intrarenal angiotensin II augmentation in angiotensin II-infused rats. *Hypertension*. 1996; 28: 669-77.

19. Kobori H, Harrison-Bernard LM and Navar LG. Urinary excretion of angiotensinogen reflects intrarenal angiotensinogen production. *Kidney Int*. 2002; 61: 579-85.

20. Yanagawa N, Capparelli AW, Jo OD, Friedal A, Barrett JD and Eggena P. Production of angiotensinogen and renin-like activity by rabbit proximal tubular cells in culture. *Kidney Int*. 1991; 39: 938-41.

21. Schulz WW, Hagler HK, Buja LM and Erdos EG. Ultrastructural localization of angiotensin I-converting enzyme (EC 3.4.15.1) and neutral metalloendopeptidase (EC 3.4.24.11) in the proximal tubule of the human kidney. *Lab Invest*. 1988; 59: 789-97.

22. Wolf G, Schneider A, Wenzel U, Helmchen U and Stahl RA. Regulation of glomerular TGF-beta expression in the contralateral kidney of two-kidney, one-clip hypertensive rats. *J Am Soc Nephrol*. 1998; 9: 763-72.

23. Wang T and Giebisch G. Effects of angiotensin II on electrolyte transport in the early and late distal tubule in rat kidney. *Am J Physiol*. 1996; 271: F143-9.

24. Barreto-Chaves ML and Mello-Aires M. Effect of luminal angiotensin II and ANP on early and late cortical distal tubule HCO3- reabsorption. *Am J Physiol*. 1996; 271: F977-84.

25. Kobori H, Harrison-Bernard LM and Navar LG. Enhancement of angiotensinogen expression in angiotensin II-dependent hypertension. *Hypertension*. 2001; 37: 1329-35.

26. Nishiyama A, Konishi Y, Ohashi N, et al. Urinary angiotensinogen reflects the activity of intrarenal renin-angiotensin system in patients with IgA nephropathy. *Nephrol Dial Transplant*. 2011; 26: 170-7.

27. Urushihara M and Kagami S. Urinary angiotensinogen as a biomarker of nephropathy in childhood. *International journal of nephrology*. 2011; 2011: 206835.

28. Kobori H, Ohashi N, Katsurada A, et al. Urinary angiotensinogen as a potential biomarker of severity of chronic kidney diseases. *J Am Soc Hypertens*. 2008; 2: 349-54.

29. Kobori H and Navar LG. Urinary Angiotensinogen as a Novel Biomarker of Intrarenal Renin-Angiotensin System in Chronic Kidney Disease. *Int Rev Thromb*. 2011; 6: 108-16.

30. Kobori H, Alper AB, Jr., Shenava R, et al. Urinary angiotensinogen as a novel biomarker of the intrarenal renin-angiotensin system status in hypertensive patients. *Hypertension*. 2009; 53: 344-50.

31. van den Heuvel M, Batenburg WW, Jainandunsing S, et al. Urinary renin, but not angiotensinogen or aldosterone, reflects the renal renin-angiotensin-aldosterone system activity and the efficacy of renin-angiotensin-aldosterone system blockade in the kidney. *J Hypertens*. 2011; 29: 2147-55.

32. Schroten NF, Gaillard CA, van Veldhuisen DJ, Szymanski MK, Hillege HL and de Boer RA. New roles for renin and prorenin in heart failure and cardiorenal crosstalk. *Heart Fail Rev*. 2011.

33. Levey AS, Coresh J, Balk E, et al. National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Annals of internal medicine*. 2003; 139: 137-47.

34. Williams SG, Ng LL, O'Brien RJ, et al. Complementary roles of simple variables, NYHA and N-BNP, in indicating aerobic capacity and severity of heart failure. *Int J Cardiol*. 2005; 102: 279-86.

35. Ceia F, Fonseca C, Mota T, et al. Aetiology, comorbidity and drug therapy of chronic heart failure in the real world: the EPICA substudy. *Eur J Heart Fail*. 2004; 6: 801-6.

36. Kung T, Szabo T, Springer J, Doehner W, Anker SD and von Haehling S. Cachexia in heart disease: highlights from the ESC 2010. *Journal of cachexia, sarcopenia and muscle*. 2011; 2: 63-9.

37. von Haehling S, Stepney R and Anker SD. Advances in understanding and treating cardiac cachexia: highlights from the 5th Cachexia Conference. *Int J Cardiol*. 2010; 144: 347-9.

38. Shapiro BA, Olala L, Arun SN, Parker PM, George MV and Bollag WB. Angiotensin II-activated protein kinase D mediates acute aldosterone secretion. *Mol Cell Endocrinol*. 2010; 317: 99-105.

39. Yamashiro T, Kuge H, Zhang J and Honke K. Calcineurin mediates the angiotensin II-induced aldosterone synthesis in the adrenal glands by up-regulation of transcription of the CYP11B2 gene. *Journal of biochemistry*. 2010; 148: 115-23.

40. Klett C and Hackenthal E. Induction of angiotensinogen synthesis and secretion by angiotensin II. *Clin Exp Hypertens A*. 1987; 9: 2027-47.

41. Klett C, Muller F, Gierschik P and Hackenthal E. Angiotensin II stimulates angiotensinogen

synthesis in hepatocytes by a pertussis toxin-sensitive mechanism. *FEBS Lett*. 1990; 259: 301-4.

42. Lynch KR and Peach MJ. Molecular biology of angiotensinogen. *Hypertension*. 1991; 17: 263-9.

43. Coezy ED, Corvol P and Howlett AC. Involvement of a pertussis toxin-sensitive G protein in the regulation of angiotensinogen production by an angiotensin II analog in HepG2 cells. *Cellular signalling*. 1990; 2: 67-76.

44. Klett CP, Printz MP, Bader M, Ganten D and Eggena P. Angiotensinogen messenger RNA stabilization by angiotensin II. *J Hypertens Suppl*. 1996; 14: S25-36.

45. Hilgenfeldt U. Half-life of rat angiotensinogen: influence of nephrectomy and lipopolysaccharide stimulation. *Mol Cell Endocrinol*. 1988; 56: 91-8.

46. Zhu JR, Cai NS, Fan WH, et al. [Efficacy and safety of olmesartan medoxomil versus losartan potassium in Chinese patients with mild to moderate essential hypertension]. *Zhonghua xin xue guan bing za zhi*. 2006; 34: 877-81.

47. Weir MR, Punzi HA, Flack JM, et al. A randomized, double-blind, forced-titration study to compare olmesartan medoxomil versus losartan potassium in patients with stage 1 and 2 hypertension. *Postgraduate medicine*. 2011; 123: 80-7.

48. Smith DH, Dubiel R and Jones M. Use of 24-hour ambulatory blood pressure monitoring to assess antihypertensive efficacy: a comparison of olmesartan medoxomil, losartan potassium, valsartan, and irbesartan. *American journal of cardiovascular drugs : drugs, devices, and other interventions*. 2005; 5: 41-50.

49. Ferreira JC, Bacurau AV, Evangelista FS, et al. The role of local and systemic renin angiotensin system activation in a genetic model of sympathetic hyperactivity-induced heart failure in mice. *American journal of physiology Regulatory, integrative and comparative physiology*. 2008; 294: R26-32.

50. De Mello WC and Frohlich ED. On the local cardiac renin angiotensin system. Basic and clinical implications. *Peptides*. 2011; 32: 1774-9.

Tables

Table 1 – Demographic characteristics of the CHF patients according to disease severity

	Mild (I-II)	Severe (III-IV)
Number of patients (n)	35	25
Age (years)	67.51 ± 11.75	74.64 ± 11.17 *
Gender (M:F)	1.23 ± 0.42	1.50 ± 0.54 *
Weight (Kg)	76.72 ± 14.42	66.81 ± 11.83*
SBP (mmHg)	123.14 ± 20.35	114.48 ± 20.21
DBP (mmHg)	69.43 ± 11.47	66.20 ± 9.26
Etiology	Ischemic (43.3%)	Ischemic (45.4%)
	Dilated (33.3%)	Dilated (40%)
	Hypertensive (20%)	Hypertensive (10%)
	Valvular (3.3%)	Valvular (10%)
ACEi treated	31 (88%)	14 (56%)
ARB treated	4 (11%)	5 (20%)

* $p<0.05$ vs mild CHF patients

Table 2 – Demographic characteristics of the CHF patients according to RAS blockade drug treatment

	ACEi-only	ARB-only
Number of patients (n)	42	6
Age (years)	68.59 ± 12.12	72.67 ± 8.04
Gender (M:F)	1.33 ± 0.47	1.50 ± 0.54
Weight (Kg)	73.49 ± 14.74	78.58 ± 8.00
SBP (mmHg)	118.38 ± 20.45	132.33 ± 16.28
DBP (mmHg)	66.76 ± 10.73	77.33 ± 7.25 *
Functional Class (NYHA)	Mild (n=29)	Mild (n=2)
	Severe (n=13)	Severe (n=4)

* $p<0.05$ vs ACEi-only treated CHF patients

Figures

Figure 1 – Plasma BNP (log pg/ml) and cystatin C (ng/l) concentrations and GFR (ml/min/1.73 m^2) in mild (n=30-35) and severe (n=21-25) CHF patients. * $p<0.05$ vs mild CHF patients.

Figure 2 – Plasma AGT (ug/ml), renin (U/l) and aldosterone (log ng/dl) concentrations and ACE activity (log U/l) in mild (n=29-33) and severe (n=21-24) CHF patients. * $p<0.05$ vs mild CHF patients.

Figure 3 - Plasma BNP (log pg/ml) and cystatin C (ng/l) concentrations and GFR (ml/min/1.73 m^2) in ACEi-only (n=36-42) and ARB-only (n=5-6) CHF patients. * $p<0.05$ vs ACEi-only treated CHF patients.

Figure 4 – Plasma AGT (ug/ml), renin (U/l) and aldosterone (log ng/dl) concentrations and ACE activity (log U/l) in ACEi-only (n=36-39) and ARB-only (n=4-6) CHF patients. * $p<0.05$ vs ACEi-only treated CHF patients.

Figure 5 – Urinary AGT excretion (log ug/g) in (A) mild (n=33) and severe (n=20) CHF patients and in (B) ACEi-only (n=38) and ARB-only (n=6) treated CHF patients.

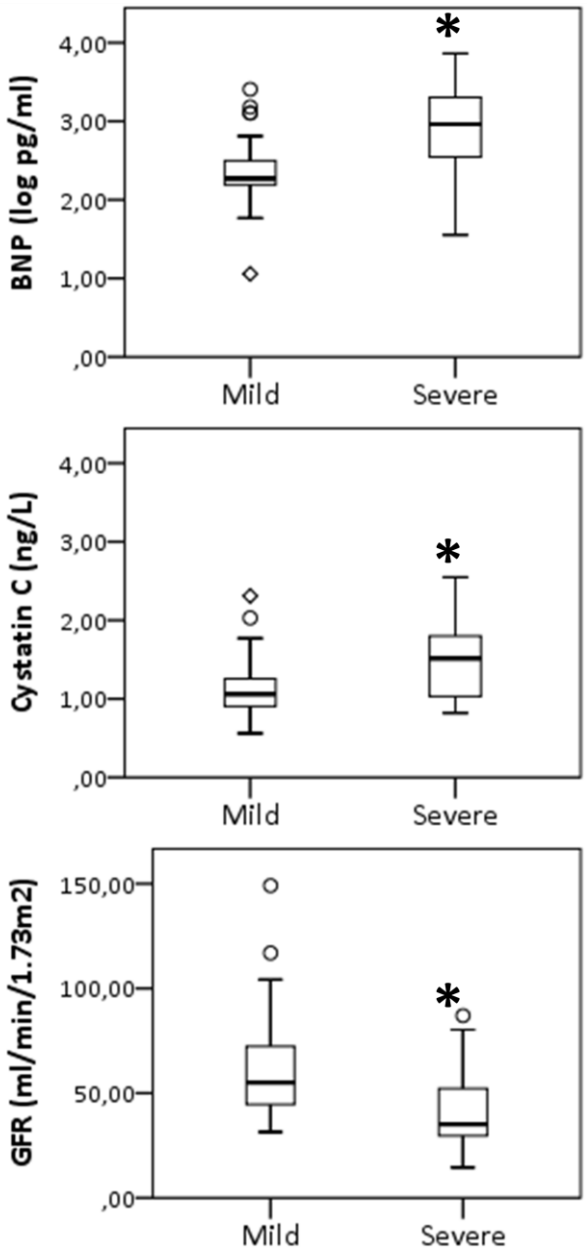
Figure 6 – Urinary AGT excretion (log ug/g) in (A) CHF patients with (n=36) and without (n= 17) CRF, (B) mild (n=18) and severe (n=17) CHF with CRF, and (C) mild (n=16) and severe (n=3) CHF patients without CRF. * $p<0.05$ vs CRF or mild groups.

Figure 7 - Plasma BNP (log pg/ml) and cystatin C (ng/l) concentrations and GFR (ml/min/1.73 m^2) in mild (n=14-16) and severe (n=2-3) CHF patients without CRF. * $p<0.05$ vs mild group.

Figure 8 - Plasma AGT (ug/ml), renin (U/l) and aldosterone (log ng/dl) concentrations and ACE activity (log U/l) in mild (n=13-15) and severe (n=2-3) CHF patients without CRF. * $p<0.05$ vs mild group.

Figure 9 - Correlation analysis between urinary excretion of AGT (log ug/g) and SBP (mmHg) (A), DBP (mmHg) (B) and plasma AGT(ug/ml) concentration (C) in CHF patients without CRF (n=17).

Figure 1


BNP (log pg/ml)
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2,00
1,00
,00
Mild
Severe
Cystatin C (ng/L)
4,00
3,00
2,00
1,00
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Mild
Severe
GFR (ml/min/1.73m2)
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100,00
50,00
,00
Mild
Severe

Figure 2

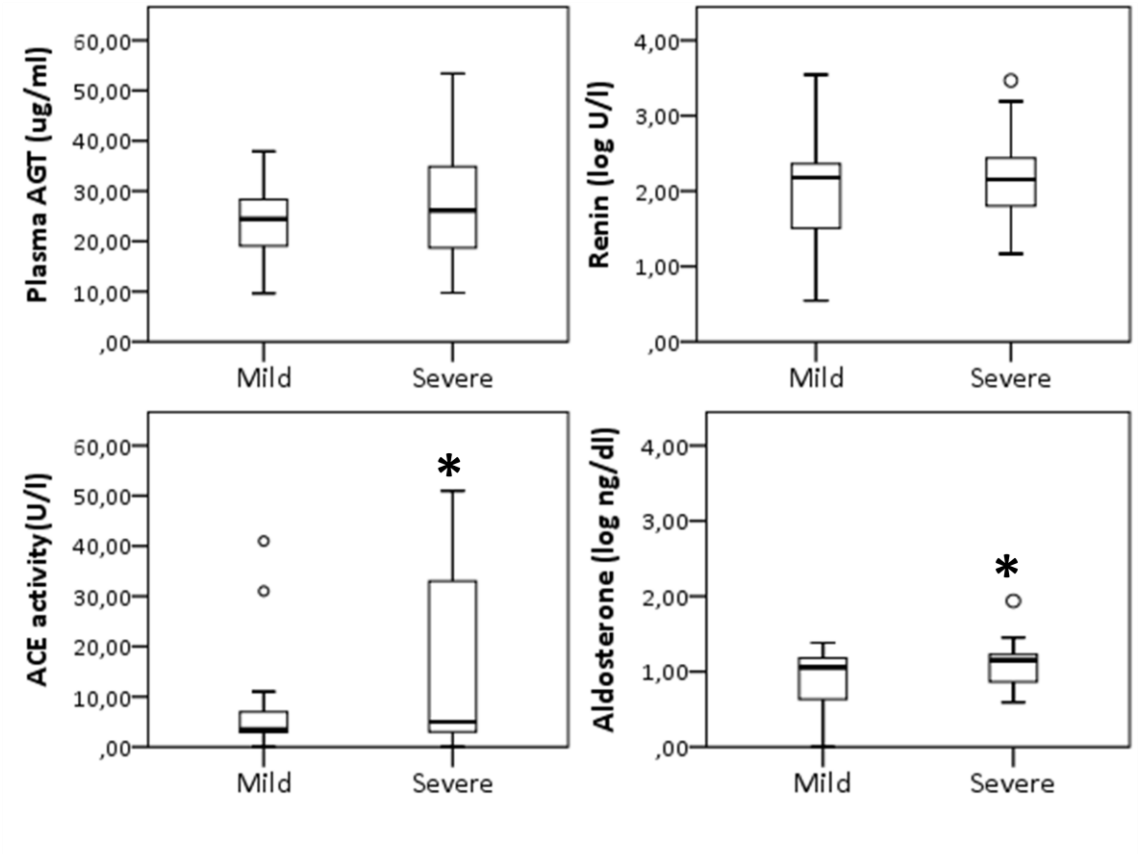
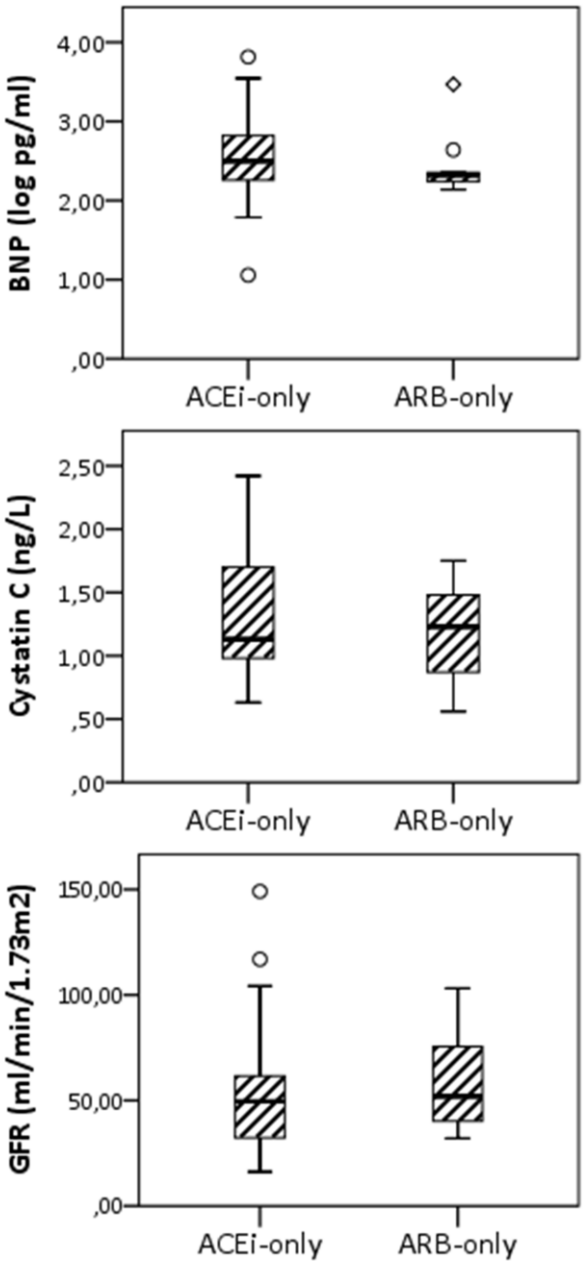

Plasma AGT (ug/ml)
60,00
50,00
40,00
30,00
20,00
10,00
,00
Mild
Severe
Renin (log U/l)
4,00
3,00
2,00
1,00
,00
Mild
Severe
ACE activity(U/l)
60,00
50,00
40,00
30,00
20,00
10,00
,00
*
Mild
Severe
Aldosterone (log ng/dl)
4,00
3,00
2,00
1,00
,00
*
Mild
Severe

Figure 3


BNP (log pg/ml)
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3,00
2,00
1,00
,00
ACEi-only
ARB-only
Cystatin C (ng/L)
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1,50
1,00
,50
,00
ACEi-only
ARB-only
GFR (ml/min/1.73m2)
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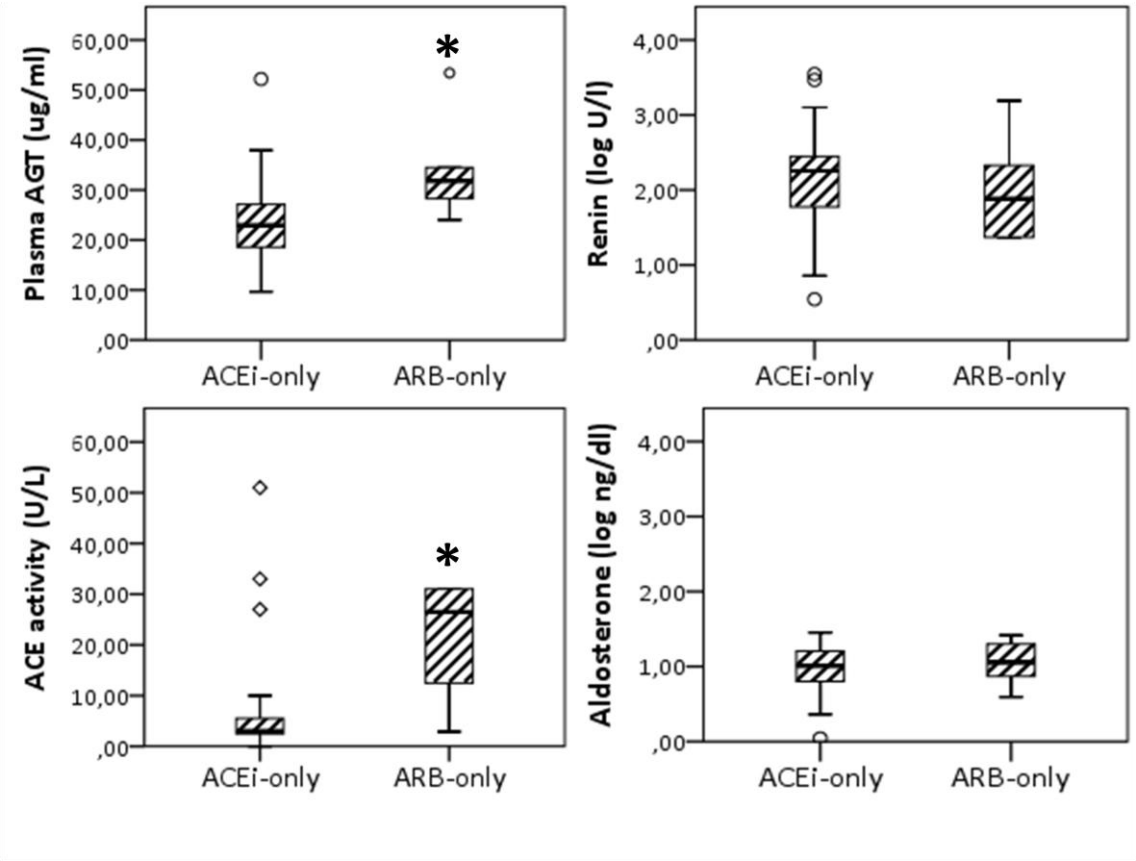
100,00
50,00
,00
ACEi-only
ARB-only

Figure 4


Plasma AGT (ug/ml)
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50,00
40,00
30,00
20,00
10,00
,00
ACEi-only
ARB-only
Renin (log U/l)
4,00
3,00
2,00
1,00
,00
ACEi-only
ARB-only
ACE activity (U/L)
60,00
50,00
40,00
30,00
20,00
10,00
,00
ACEi-only
ARB-only
Aldosterone (log ng/dl)
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2,00
1,00
,00
ACEi-only
ARB-only

Figure 5

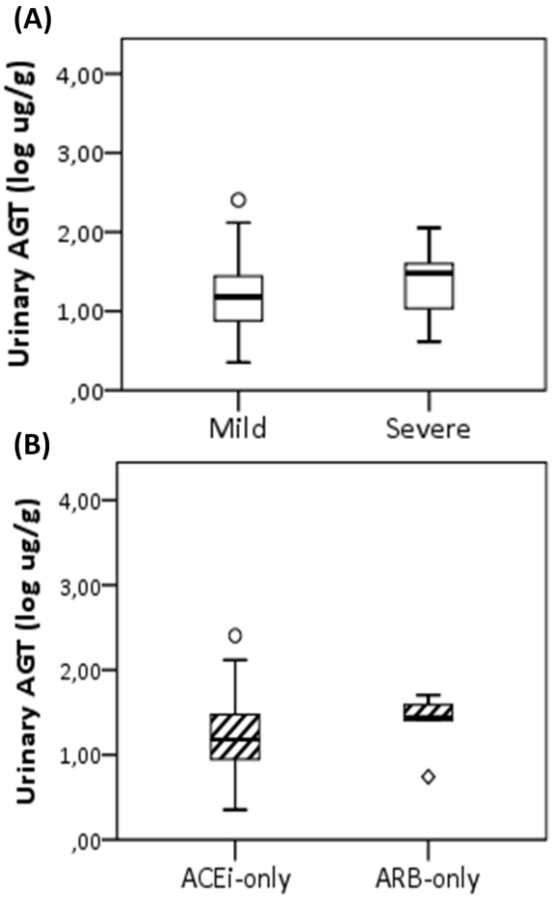
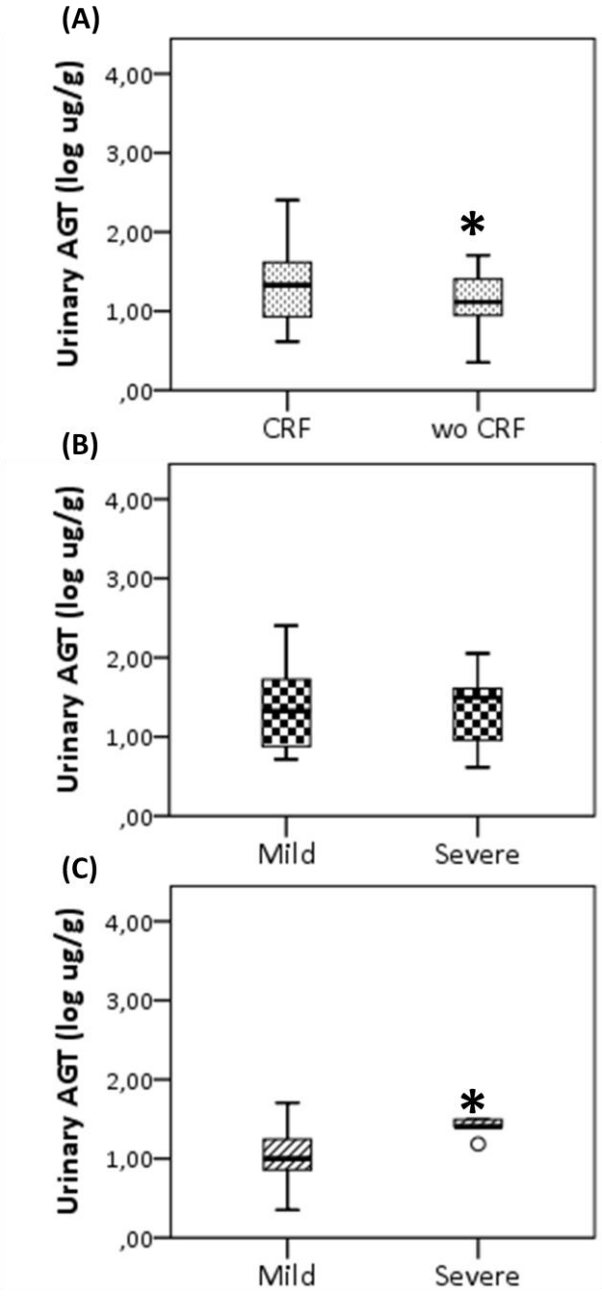

(A)
Urinary AGT (log ug/g)
4,00
3,00
2,00
1,00
,00
Mild
Severe
(B)
Urinary AGT (log ug/g)
4,00
3,00
2,00
1,00
,00
ACEi-only
ARB-only

Figure 6


(A)
Urinary AGT (log ug/g)
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3,00
2,00
1,00
,00
CRF
wo CRF
(B)
Urinary AGT (log ug/g)
4,00
3,00
2,00
1,00
,00
Mild
Severe
(C)
Urinary AGT (log ug/g)
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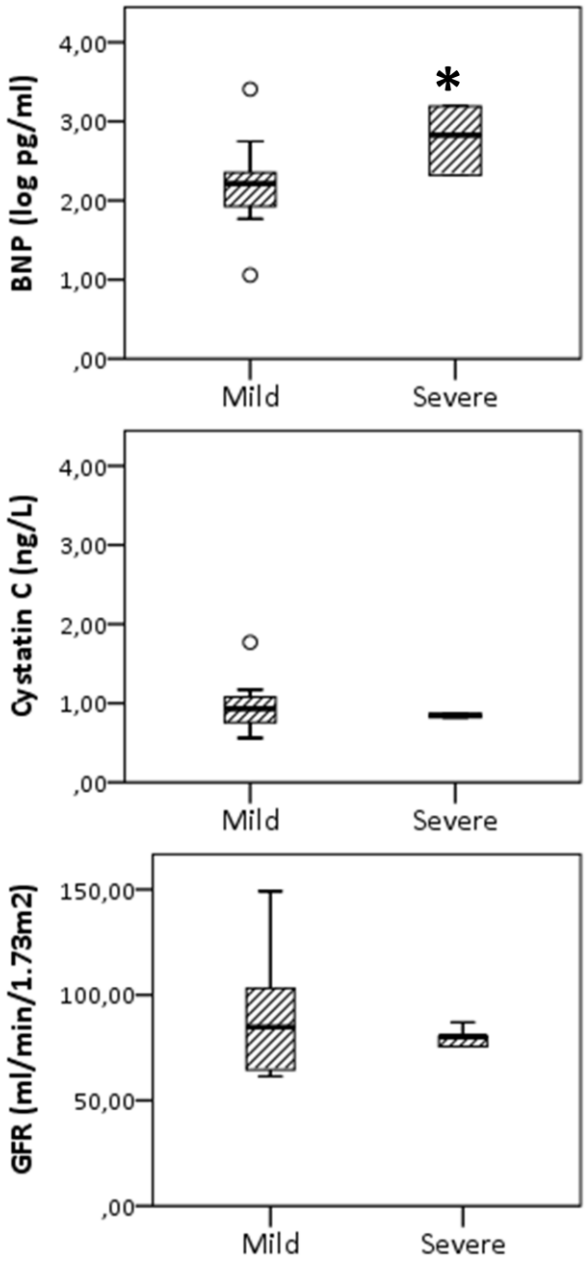
3,00
2,00
1,00
,00
Mild
Severe

Figure 7


BNP (log pg/ml)
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3,00
2,00
1,00
,00
*
Mild
Severe
Cystatin C (ng/L)
4,00
3,00
2,00
1,00
,00
Mild
Severe
GFR (ml/min/1.73m2)
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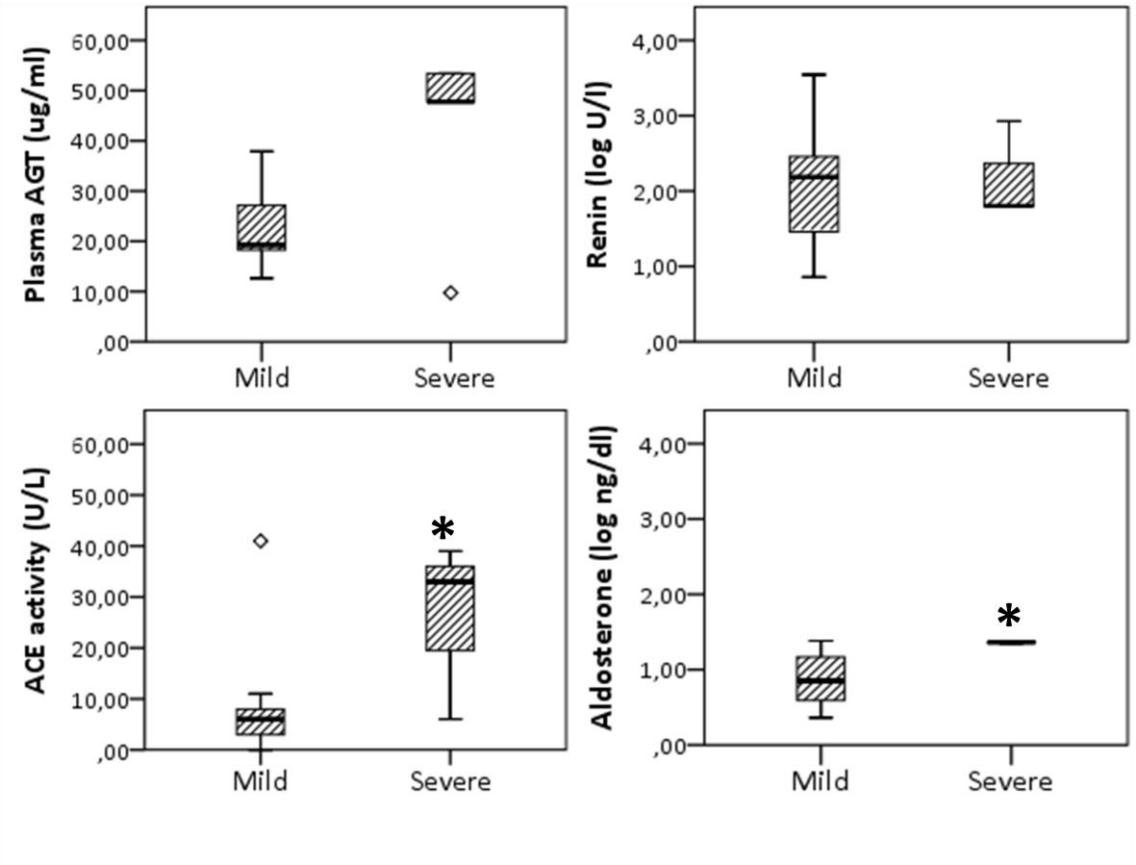
100,00
50,00
,00
Mild
Severe

Figure 8


Plasma AGT (ug/ml)
60,00
50,00
40,00
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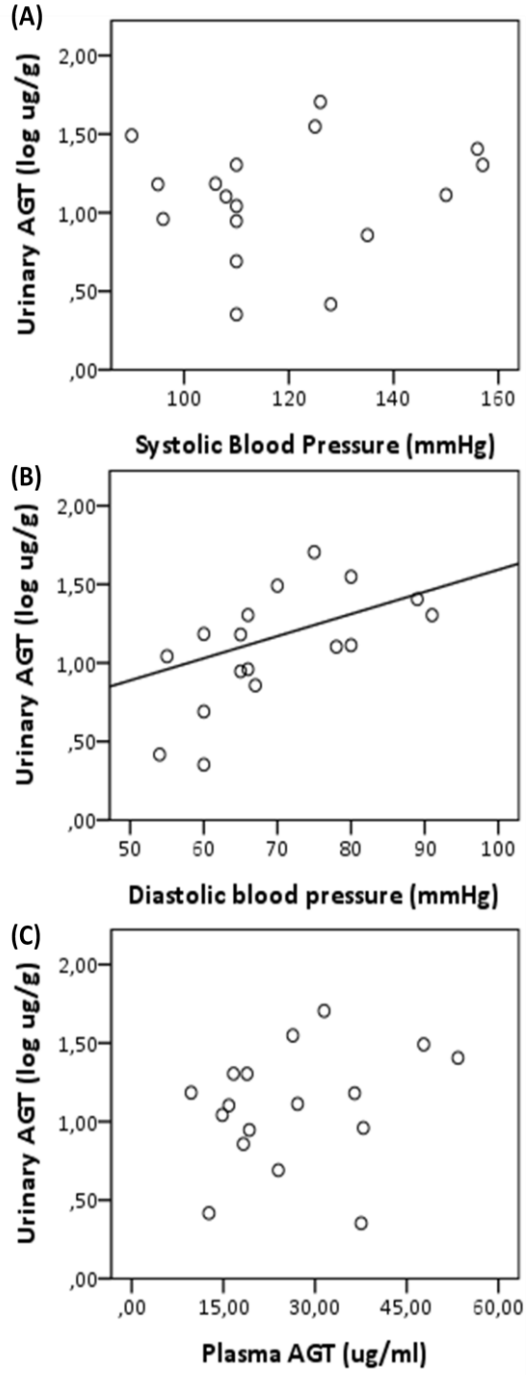
20,00
10,00
,00
Mild
Severe
Renin (log U/l)
4,00
3,00
2,00
1,00
,00
Mild
Severe
ACE activity (U/L)
60,00
50,00
40,00
30,00
20,00
10,00
,00
*
Mild
Severe
Aldosterone (log ng/dl)
4,00
3,00
2,00
1,00
,00
*
Mild
Severe

Figure 9


(A)
Urinary AGT (log ug/g)
2,00
1,50
1,00
,50
,00
100
120
140
160
Systolic Blood Pressure (mmHg)
(B)
Urinary AGT (log ug/g)
2,00
1,50
1,00
,50
,00
50
60
70
80
90
100
Diastolic blood pressure (mmHg)
(C)
Urinary AGT (log ug/g)
2,00
1,50
1,00
,50
,00
,00
15,00
30,00
45,00
60,00
Plasma AGT (ug/ml)

Anexo 1

Manuscript Submission Guidelines:

Journal of the Renin-Angiotensin-Aldosterone System

1. Peer review policy

2. Article types

2.1 Summary of manuscript structure/style

3. How to submit your manuscript

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10.2 E-Prints and complimentary copies

10.3 SAGE production

10.4 OnlineFirst publication

11. Further information

The *Journal of the Renin-Angiotensin-Aldosterone System* is a peer-reviewed journal published quarterly as a resource for biomedical professionals, including basic scientists and clinicians, primarily with an active interest in the renin-angiotensin-aldosterone system in humans and other mammals.

It publishes original research articles and reviews on the normal and abnormal function of this system and its pharmacology and therapeutics, mostly in a cardiovascular context but including research in all areas where this system is present, including the brain, lungs and gastro-intestinal tract.

Although this is its main focus, JRAAS also publishes research on other peptides, such as vasopressin, the natriuretic peptides and the kallikrein-kinin system.

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The journal's policy is to obtain at least two independent reviews of each article. It operates a double-blind reviewing policy in which the reviewer‟s name is always concealed from the submitting author; authors may choose to reveal their name but the journal otherwise leaves the article anonymous. Referees will be encouraged to provide substantive, constructive reviews that provide suggestions for improving the work and distinguish between mandatory and non-mandatory recommendations.

All manuscripts accepted for publication are subject to editing for presentation, style and grammar. Any major redrafting is agreed with the author but the Editor's decision on the text is final.

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Journal of the Renin-Angiotensin-Aldosterone System is pleased to consider original papers, review articles and letters for publication. All material is assumed to be exclusively submitted to JRAAS unless otherwise stated in writing.

Manuscripts are considered for publication with the understanding that they have not been published previously and are not under consideration by another publication.

2.1 Summary of manuscript structure:

Review articles provide in-depth surveys of recent advances in a field. Suggestions for review articles should be provided in the form of a one page synopsis or discussed with the editors prior to submission.

Original articles may appear as full length reports (approximately 5,000 words excluding references and figure legends) or short communication (approximately 1,500 words) which report preliminary data from original work.

Letters are encouraged to provide comments on previously published papers or on important or novel aspects of research in the field.

Review manuscripts should include an abstract (150-200 words) and keywords.

Original manuscripts should include: Title page, Abstract (150-200 words, see below) plus keywords, Introduction, Materials and methods, Results, Discussion, Conclusions, References (Vancouver style), Tables, Figures, Acknowledgements.

Abstracts for original manuscripts should be in the format: Hypothesis / Introduction, Materials and methods, Results, Conclusions.

Illustrations, artwork and photographs.

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Review your submission (in both PDF and HTML formats) and then click the Submit button

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Complete anonymity is difficult to achieve, however, and informed consent should be obtained if there is any doubt. For example, masking the eye region in photographs of patients is inadequate protection of anonymity. If identifying characteristics are altered to protect anonymity, such as in genetic pedigrees, authors should provide assurance that alterations do not distort scientific meaning and editors should so note.

When informed consent has been obtained it should be indicated in the submitted article.

Authors should identify individuals who provide writing/administrative assistance, indicate the extent of assistance and disclose the funding source for this assistance.

Identifying details should be omitted if they are not essential.

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When reporting experiments on human subjects, indicate whether the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) or with the Declaration of Helsinki 1975, revised Hong Kong 1989. Do not use patients' names, initials or hospital numbers, especially in illustrative material. When reporting experiments on animals, indicate which guideline/law on the care and use of laboratory animals was followed.

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Any acknowledgements should appear first at the end of your article prior to your Declaration of Conflicting Interests (if applicable), any notes and your References.

All contributors who do not meet the criteria for authorship should be listed in an `Acknowledgements‟ section. Examples of those who might be acknowledged include a person who provided purely technical help, writing assistance, or a department chair who provided only general support. Authors should disclose whether they had any writing assistance and identify the entity that paid for this assistance.

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To comply with the guidance for Research Funders, Authors and Publishers issued by the Research Information Network (RIN), *Journal of the Renin-Angiotensin-*

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9. Manuscript style

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9.2 Journal Style

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The text should be double-spaced throughout and with a minimum of 3cm for left and right hand margins and 5cm at head and foot. Text should be standard 10 or 12 point.

9.4.1 Keywords and Abstracts: Helping readers find your article online

The title, keywords and abstract are key to ensuring that readers find your article online through online search engines such as Google. Please refer to the information and guidance on how best to title your article, write your abstract and select your keywords by visiting SAGE‟s Journal Author Gateway Guidelines on How to Help Readers Find Your Article Online.

9.4.2 Corresponding Author Contact details

Provide full contact details for the corresponding author including email, mailing address and telephone numbers. Academic affiliations are required for all co-authors.

9.4.3 Guidelines for submitting artwork, figures and other graphics

For guidance on the preparation of illustrations, pictures and graphs in electronic format, please visit SAGE‟s Manuscript Submission Guidelines.

Images should be supplied as bitmap based files (i.e. with .tiff or .jpeg extension) with a resolution of at least **300 dpi** (dots per inch). Line art should be supplied as vector-based, separate .eps files (not as .tiff files, and not only inserted in the Word or pdf file), with a resolution of **600 dpi**. Images should be clear, in focus, free of pixilation and not too light or dark.

If, together with your accepted article, you submit usable colour figures, these figures will appear in colour online regardless of whether or not these illustrations are reproduced in colour in the printed version. For specifically requested colour reproduction in print, you will receive information regarding the possible costs from SAGE after receipt of your accepted article.

In text: tables and figures are either inserted as part of a sentence, for example table 1 or in parentheses for example (figure 1). Each table should carry a descriptive heading. Each figure should be submitted electronically.

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The journal may be able to host approved supplemental materials online, alongside the full-text of articles. Supplemental files will be subjected to peer-review alongside the article. Please contact the Editor (m.schachter@imperial.ac.uk) in the first instance. For more information please refer to SAGE‟s Guidelines for Authors on Supplemental Files.

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We will email a PDF of the proofs to the corresponding author. Corrections should be limited to typographical amendments. Authors' approval will be assumed if corrections are not returned by the date indicated.

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11. Further information

If you would like to discuss your paper prior to submission contact the Editor:

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