

BRIEF REPORT

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# Stronger association of intact angiotensinogen with mortality than lactate or renin in critical illness: post-hoc analysis from the VICTAS trial

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## Abstract

Sepsis and septic shock remain global healthcare problems associated with high mortality rates despite best therapy efforts. Circulating biomarkers may identify those patients at risk for poor outcomes, however, current biomarkers, most prominently lactate, are non-specific and have an inconsistent impact on prognosis and/or disease management. Activation of the renin-angiotensin- system (RAS) is an early event in sepsis patients and elevated levels of circulating renin are more predictive of worse outcomes than lactate. The precursor protein Angiotensinogen is another key component of the circulating RAS; it is the only known substrate for renin and the ultimate source of the vasopressor Angiotensin II (Ang II). We postulate that lower Angiotensinogen concentrations may reflect a dysfunctional RAS characterized by high renin concentrations but attenuated Ang II generation, which is disproportionate to the high renin response and may compromise adequate support of blood pressure and tissue perfusion in septic patients. The current study compared the association between serum Angiotensinogen with mortality to that of lactate and renin in the VICTAS cohort of sepsis patients at baseline (day 0) by receiver operating characteristic (ROC) and Kaplan–Meier curve analyses. Serum concentration of Angiotensinogen was more strongly associated with 30-day mortality than either the serum concentrations of renin or lactate in sepsis patients. Moreover, the clinical assessment of Angiotensinogen may have distinct advantages over the typical measures of renin. The assessment of intact Angiotensinogen may potentially facilitate more precise therapeutic approaches (including exogenous angiotensin II) to restore a dysfunctional RAS and improve patient outcomes. Additional prospective validation studies are clearly required for this biomarker in the future.

**Keywords** Sepsis, Septic shock, Outcomes, Biomarkers, Renin, Lactate, Angiotensinogen

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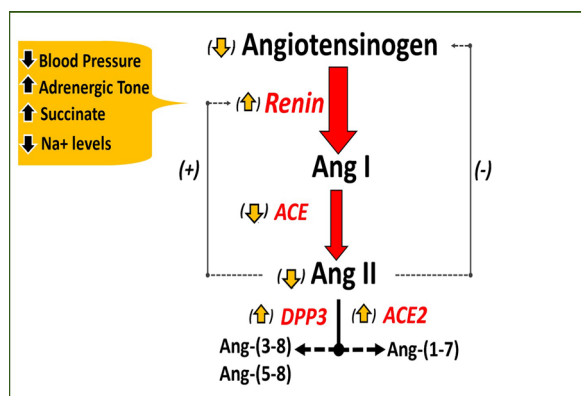


## Introduction

Sepsis and septic shock remain global healthcare problems associated with mortality rates of up to 40% despite best care efforts in the millions of patients affected every year. Circulating markers of sepsis severity may identify those patients at risk for poor outcomes early in the course of the disease; however, currently used disease biomarkers, most prominently lactate, are non-specific and have an inconsistent impact on prognosis and/or disease management [1]. Activation of the renin–angiotensin–aldosterone system (RAS) is an early event in sepsis and elevated levels of circulating renin, a protease that activates the RAS cascade, is more predictive of worse outcomes in septic patients than lactate and may be a relevant biomarker in guided therapy [2–5]. Various conditions associated with sepsis and/or septic shock may stimulate the release of renin from the kidney including a reduction in blood pressure, higher adrenergic tone, increased circulating levels of succinate and lower tubular fluid Na<sup>+</sup> content (Fig. 1) [6–8]. Apart from an increase in renin which, other aspects of an altered RAS in sepsis include lower levels of angiotensin converting enzyme (ACE), reduced expression or responsiveness of the Ang II type 1 receptor (AT<sub>1</sub>R), and a lower serum ratio of Ang II/Ang I. The precursor protein angiotensinogen that is primarily released by the

liver into the blood is another key component of the circulating RAS; it is the only known substrate for renin and the ultimate source for angiotensin peptides including the downstream vasopressor product angiotensin II (Ang II). Although angiotensinogen is typically thought to circulate at saturating concentrations with respect to renin, the concentrations of the precursor in humans approximate the optimal kinetic value for renin (Michaelis–Menten constant or Km of 1000 nM), such that changes in the circulating levels of angiotensinogen may impact the subsequent generation of Ang II [9, 10]. Indeed, siRNA-based approaches that chronically downregulate expression of angiotensinogen are promising antihypertensive therapies by reducing the formation of Ang II and the subsequent activation of downstream signaling event through the AT<sub>1</sub> receptor (AT<sub>1</sub>R) [10].

We previously reported the association of elevated renin content to disease severity in the VICTAS cohort (Vitamin C, Thiamine and Steroids in Sepsis trial) [5]. Here we saw that the Ang II response was disproportionate to the high renin levels in those patients who exhibited increased mortality [5, 6]. We now extend our previous work, with the postulation that lower circulating concentrations of angiotensinogen in sepsis and septic shock patients may reflect a dysfunctional RAS characterized by high renin levels, but an attenuated generation of Ang II [2–6]. A blunted Ang II response may be detrimental in critically-ill patients regarding the inability to maintain adequate blood pressure and tissue perfusion [3, 5, 6, 8].



**Fig. 1** Dysregulation of the RAS in sepsis and septic shock. Circulating angiotensinogen is processed by renin to angiotensin I (Ang I), which is immediately converted to Ang II by ACE. Ang II is metabolized by dipeptidyl peptidase 3 (DPP3) to Ang-(3–8) and Ang-(5–8) while ACE2 converts Ang II to Ang-(1–7). Ang II inhibits the release of renin while Ang II stimulates the release of angiotensinogen. In sepsis and septic shock, lower blood pressure and tubular Na<sup>+</sup> but higher adrenergic tone and succinate stimulate renin release. Reduced levels of ACE but higher DPP3 and ACE2 contribute to blunted Ang II levels despite increased renin levels. Reduced Ang II and AT<sub>1</sub> receptor (AT<sub>1</sub>R) responsiveness, as well as high renin levels may lead to lower circulating angiotensinogen, which may further depress the generation of Ang II. Lower Ang II and AT<sub>1</sub>R may also stimulate the release of renin. [6] Adapted from Schaich et al.

## Methods

The current post-*hoc* study of the VICTAS cohort (Vitamin C, Thiamine and Steroids in Sepsis trial) compared the association between circulating levels of intact angiotensinogen with mortality compared to lactate and active renin in a subset of sepsis and septic shock patients at baseline (day 0, N = 103). Intact human angiotensinogen, which contains the angiotensin I (Ang I) domain released by renin and subsequently converted to Ang II by ACE was determined by a human Enzyme-Linked Immunosorbent Assay (ELISA) (IBL America, Minneapolis MN USA). The thawed samples on ice were pretreated with the specific renin inhibitor aliskerin (1 μM, MedChem, Monmouth NJ, USA) to stabilize the intact protein during the assay. Active renin protein was measured by a human renin ELISA (DRG International Fisher Scientific, Waltham MA, USA) that recognizes the active site of renin as described [5]. Serum lactate was determined by standard methods [3]. Serum biomarker discrimination for all-cause, 30-day mortality was compared graphically via receiver operating characteristic (ROC) curves generated by multivariable logistic regression models

adjusted for age, sex, sequential organ failure assessment (SOFA) score, systolic blood pressure, and in-hospital vasopressor administration. Area under the curve (AUC) for angiotensinogen and renin was compared to lactate (DeLong's test). The association of angiotensinogen, renin, and lactate with mortality was assessed by Kaplan–Meier curves with log rank test and hazard ratios derived from Cox regression. Analyses were completed in R 4.2.3 (2023) with a 2-sided  $\alpha=0.05$ . Additionally, the Youden index was calculated for these biomarkers.

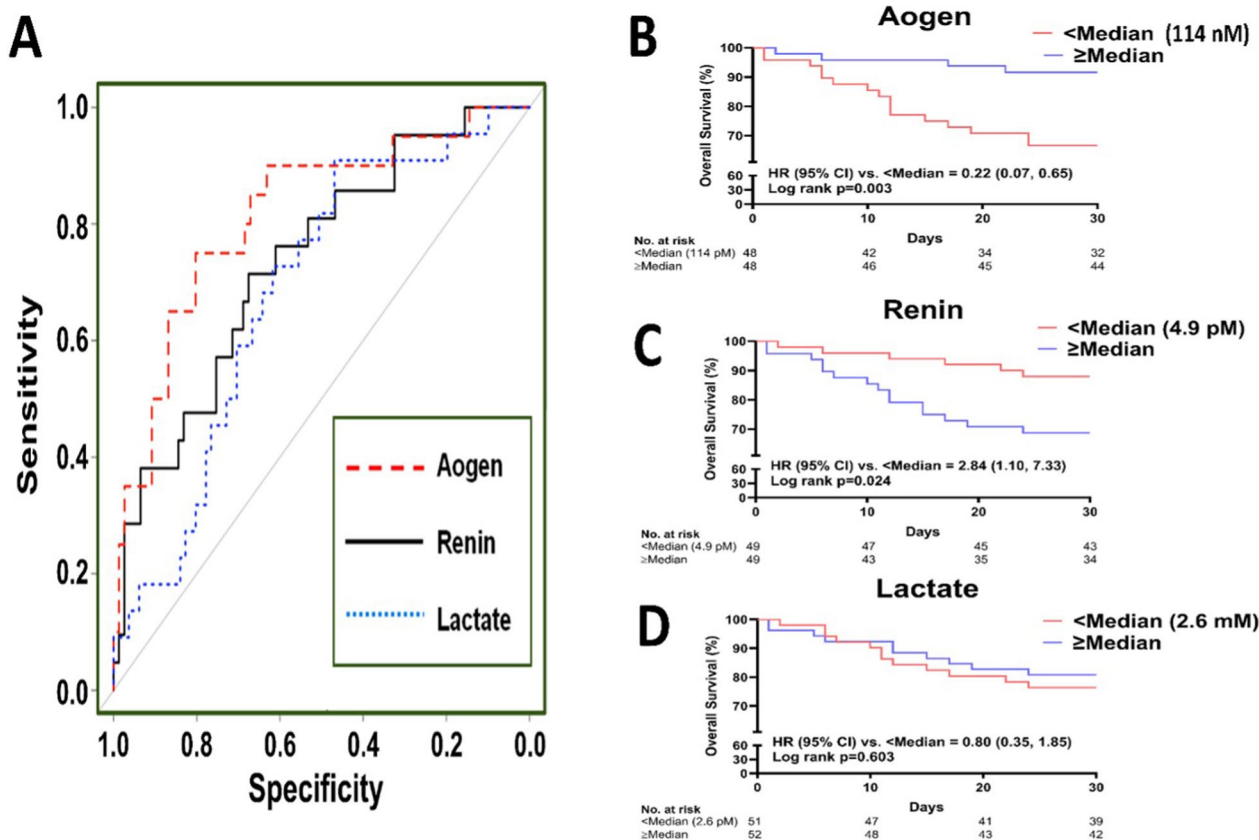
## Results

The patient characteristics for this cohort are included in the supplemental table (Table S1). Note the VICTAS study lacks patient data on use or type of RAS blockers. The median [interquartile range] serum concentrations were 114 nM [78.2–205.4] for intact angiotensinogen, 4.9 pM [2.2–13.6] for active renin and 2.6 mM [1.7–3.8] for lactate. The ROC curves revealed better discrimination of angiotensinogen for overall 30-day mortality than either active renin or lactate (Fig. 2A). The AUC of

angiotensinogen (0.82, 95% CI: 0.70, 0.93) was significantly greater than that of lactate (AUC=0.68, 95% CI: 0.57, 0.80;  $p=0.022$ ). Kaplan–Meier curves and log rank test also revealed that angiotensinogen concentrations lower than the median value of 114 nM were associated with reduced survival (Fig. 2B). Renin concentrations greater than the median value of 4.9 pM were also associated with higher patient mortality albeit less than angiotensinogen, while serum lactate concentrations (2.6 mM median value) were not associated with mortality in this cohort of patients (Fig. 2C–D). Finally, the Youden index for angiotensinogen (0.59; optimal threshold=116.2 nM) was higher than renin (0.36, optimal threshold=4.1 pM) or lactate (0.41; optimal threshold=2.3 mM).

## Discussion

The liver is the predominant source of circulating angiotensinogen and perhaps other tissues and receives ~25% of cardiac output, as well as constitutes an important site for bacterial clearance and innate immune regulation [11–13]. SOFA scores indicated no overt organ damage



**Fig. 2** Panel A: ROC curves reveal better discrimination of circulating angiotensinogen (Aogen; AUC=0.82 [0.70, 0.93]) for mortality than serum lactate (AUC=0.68 [0.57, 0.80]) or active renin (AUC=0.73 [0.61, 0.86]) in the VICTAS cohort of sepsis patients at baseline (day 0, N=103). Panels B–D: Kaplan–Meier estimates of survival curves reveal a stronger association of serum levels of Aogen (log rank  $p=0.003$ ) with mortality than renin (log rank  $p=0.024$ ) or lactate (log rank  $p=0.603$ ) in the VICTAS cohort at baseline (day 0)

in this cohort suggesting other mechanisms may negatively impact angiotensinogen expression rather than liver injury per se [5]. Downregulation of the AT<sub>1</sub> receptor (AT<sub>1</sub>R) is evident in sepsis and reduced ACE activity coupled to enhanced metabolism by ACE2 and DPP3 to generate the angiotensin metabolites angiotensin-(1–7) and angiotensin-(3–8)/(5–8) may further contribute to a blunted Ang II response (Fig. 1) [5–8, 14]. Angiotensin II is a positive regulator of liver angiotensinogen expression via stimulation of the AT<sub>1</sub>R [15, 16]. In septic patients, a lower Ang II response or reduced AT<sub>1</sub>R responsiveness may attenuate release of the precursor, particularly in the presence of high circulating renin that would deplete intact angiotensinogen levels. These conditions may further depress Ang II potentially establishing a negative feedback loop for the expression of angiotensinogen (Fig. 1). Additional investigation of the RAS axis is necessary to elucidate the precise regulation of angiotensinogen in sepsis and septic shock patients.

In this well-characterized, multisite cohort of sepsis and septic shock patients, circulating angiotensinogen was more strongly associated with 30-day mortality than either renin or lactate. Lower angiotensinogen content may reflect increased consumption by the high levels of renin, reduced expression of the precursor by liver hepatocytes or both conditions that ultimately attenuate Ang II-AT<sub>1</sub>R tone to adequately support tissue perfusion and maintain hemodynamics in critically-ill patients, as well as preserve an immune response required for bacterial clearance [3, 5, 6, 17]. The clinical assessment of angiotensinogen may have advantages over renin, particularly as the plasma renin activity (PRA) assay utilizes endogenous angiotensinogen as substrate, and the depletion of intact angiotensinogen may underestimate active renin levels. Prorenin, the inactive precursor that is more abundant in the circulation than renin by up to tenfold, can undergo cryoactivation by improper sample handling that may inflate renin activity or renin content. PRA is also a multi-step assay requiring Ang I measurement and is not suitable as a rapid point-of-care test. The reduced levels of intact angiotensinogen coupled to high active renin content may be indicative of a blunted Ang II response in critically-ill patients. In this regard, the rapid analysis of circulating Ang II in human plasma by direct ELISAs is not presently feasible given evidence of non-specificity of these assays [18]. The relevance of this work is particularly significant knowing the availability of exogenous synthetic Ang II as an approved vasopressor by the United States Food and Drug Agency (FDA) and the European Medicines Agency (EMA). The pivotal Angiotensin II in High Output Shock (ATHOS3) trial demonstrated a significant decrease of elevated renin in patients randomized to receive Ang II compared with

placebo which included standard of care vasopressors [19]. When further examining patients with a higher than population median renin level, those who received Ang II had a survival benefit. Our work is limited by the fact that the current data are “hypothesis generating” in that it is a post-*hoc* sample from a larger cohort of septic patients and that an external validation cohort to confirm the angiotensinogen response is required. Indeed, future studies investigating the prospective assessment of intact angiotensinogen may facilitate more precise therapeutic approaches to restore a dysfunctional ACE-Ang II-AT<sub>1</sub>R axis of the RAS and improve overall mortality and organ system failure in critically-ill patients [3, 20].

#### Abbreviations

ACE	Angiotensin converting enzyme
Ang II	Angiotensin II
ELISA	Enzyme-linked immunosorbent assay
PRA	Plasma renin activity
RAS	Renin angiotensin system
SOFA	Sequential organ failure assessment
VICTAS	Vitamin C, Thiamine and Steroids in Sepsis study

#### Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13054-024-05120-w>.

Additional file 1 (DOCX 16 KB)

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#### Author contributions

MCC: Conceptualization, writing, reviewing, and editing. CLS: Conceptualization, writing, reviewing, and editing. LWB: Writing, reviewing, and editing. GSM: Writing, reviewing, and editing. JES: Writing, reviewing, and editing. JKH: Writing, reviewing, and editing. AKK: Conceptualization, writing, reviewing, and editing.

#### Availability of data and materials

No datasets were generated or analysed during the current study.

#### Declarations

##### Ethics approval and consent to participate

The current study was reviewed and approved in April of 2021 with a waiver of consent by the Wake Forest University School of Medicine institutional review board (00073908) and all procedures were followed in accordance with local ethics standards and the Helsinki Declaration of 1975.

##### Competing interests

AKK has previously received consulting fees from La Jolla Pharma, including grant funding for the ATHOS3 trial. He has also previously received consulting fees from Innoviva Therapeutics and Viatrix Pharmaceuticals. The other authors declare that there are no competing financial interests in the work described in the present study.

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