Peer Review File

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REVIEWER A

REVIEWER COMMENT 1:

This review on the concept of critical illness-related mineralocorticoid insufficiency would benefit handles on an interesting topic, but could be much improved by a thorough revision of the content of the manuscript. The authors should rethink which information should be placed in which section, as now, the text is convoluted, sometimes unlogic in the order on how information is presented and therefore sometimes difficult to follow.

RESPONSE

Thank you for the comment. We have revised the content as suggested. A number of organisational changes have been made in the manuscript taking into account comments from the other two Reviewers. We also note Reviewer B's comment that "Overall, this review is well written and pleasant to read. The work well organized and comprehensively described with appropriate and adequate references."

CHANGES IN TEXT: Not applicable for this comment. However, there are a number of point by point responses to comments raised by the other Reviewers that improve the organisation of the manuscript and which apply to this comment. Please also kindly find below point by point responses to further specific points raised.

REVIEWER COMMENT 2:

Line 83-90: Abstract: the conclusion statement is very vague and should be more to the point of what was described.

RESPONSE 2

We have modified the conclusion of the abstract as advised. It now reads as follows:

Conclusion:

Difficulties are encountered in interpreting measures of gluco- and mineralo-corticoid activity in critical illness. ~~A number of pathophysiological similarities and differences between glucocorticoid and mineralocorticoid dysfunction in critical illness require exploration with an emphasis on clinical characteristics and diagnostic challenges associated with hyperreninaemic hypoaldosteronism and critical illness-related corticosteroid insufficiency.~~ Aldosterone levels, like cortisol, have been shown to be increased in sepsis and hemorrhagic shock. The finding of hyperreninemia and hyperaldosteronism with an aldosterone/plasma renin activity ratio below 2 should

prompt consideration of hyperreninemic hypoaldosteronism, a finding, which likely signifies the loss of negative feedback control of the renin-angiotensin-aldosterone system. As there is evidence to suggest that in acute critical illness, hyperreninemic hypoaldosteronism, is associated with poor outcomes, co-administration of hydrocortisone with fludrocortisone in patients with septic shock should be considered.

In keeping with the concept of critical illness-related corticosteroid insufficiency, we suggest the term critical illness-related mineralocorticoid insufficiency as a more appropriate description of the impaired aldosterone response to increased levels of renin seen in this group of patients.

CHANGES IN TEXT: Section: **Abstract.** Pages 4 and 5, line 82-96

REVIEWER COMMENT 3

Line 110 background: References to back the following statement are lacking: "the recent demonstration of significant mortality improvements associated with adjunctive glucocorticoid treatment in combination with fludrocortisone in septic shock"?

Line 114 background: Similarly, also for the statement "the suggestion that angiotensin II is effective in treating vasodilatory shock" misses proper reference to available literature.

RESPONSE 3

References have been added and the text now reads as follows for line 110 and line 114 on the old manuscript:

In this interpretative review, we consider hyperreninemic hypoaldosteronism, a concept worth re-examining given the recent demonstration of significant mortality improvements associated with adjunctive glucocorticoid treatment in combination with fludrocortisone in septic shock.(12) The suggestion that angiotensin II is effective in treating vasodilatory shock further highlights the potential role of therapeutic approaches targeting the renin-angiotensin-aldosterone system in septic shock.(13)

CHANGES IN TEXT Page 6, line 114-116 and Page 5 line 116-118

REVIEWER COMMENT 4

- Line 145-160 basic physiology: this paragraph is about basic physiology, but misses information on the basic stimulators of aldosterone synthesis and secretion.

RESPONSE

We thank the reviewer for this comment. Indeed, we elected not to include extensive basic physiology as we felt it detracted from the objective of the narrative review. We

agree the title is perhaps misleading and have thus modified the section title to read as follows:

Selected basic physiology

CHANGES IN TEXT Section: **Selected Basic Physiology**. Page 7, line 146

REVIEWER COMMENT 5

- Line 160 basic physiology: the last sentence is out place, no derangements have yet been discussed.

RESPONSE:

We thank the reviewer for this comment. This was a general statement suggesting the treatment of derangements of any kind should consider the acuity of any changes. This statement is not referring to any specific prior derangements discussed as yet. The paragraph before the statement was a discussion on effects of corticotrophin in the acute setting and how they differ to chronic corticotrophin effects.

CHANGES IN TEXT:

Not applicable for this comment. The statement in question is now on page 9, line 194 to 196

REVIEWER COMMENT 6

Furthermore, the risk of chronic corticotrophin stimulation is low during critical illness as patients typically display suppressed corticotropin levels, due to feedback of cortisol on the pituitary.

RESPONSE:

Indeed, this is correct. Multiple studies have shown that corticotrophin levels are low in acute critical illness due to a number of factors including negative feedback inhibition as a result of high free cortisol levels.[1] This has been demonstrated up to day 28. Importantly in one study, even when cortisol levels begin to decrease if illness persists beyond 4 weeks, ACTH did not rise until later when patients were in the ward setting (out of ICU). This suggests that negative feedback inhibition from high free cortisol may not be the sole mechanism behind low ACTH levels seen in critical illness and that central suppression from another mechanism may play a role. This is indeed supported by the fact that with the ACTH rise seen later on, recovery was accompanied by elevation in free cortisol levels even though the acute illness had subsided. This discussion in this section of the paper is, however, meant to merely highlight the opposing effects of acute and chronic ACTH.

However, the low ACTH seen in acute critical illness is of particular interest with regards to CIRMI as experimental evidence suggests that ACTH is required for normal aldosterone production.[2] This is supportive of the concept of CIRMI as yet another mechanism which links derangements in cortisol physiology during critical

illness to derangements in aldosterone physiology.

For further clarification we have added a paragraph in the section to clarify the link between the low ACTH levels seen in critical illness as follows:

Corticotrophin levels are typically low in acute critical illness due to factors such as negative feedback inhibition as a result of high free cortisol levels seen in critical illness.(41) This has been demonstrated up to day 28 of illness. Importantly in one study, even when cortisol levels begin to decrease if illness persists beyond 4 weeks, corticotrophin levels did not rise until later during recovery.(41) The low corticotrophin seen in acute critical illness is of interest as experimental evidence suggests that corticotrophin is required for normal aldosterone production.(42) Reduced levels of aldosterone have been shown in a pro-opiomelanocortin-knockout mouse model, suggesting a mechanism through which derangements in cortisol physiology are associated with derangements in aldosterone physiology during critical illness.(42)

CHANGES IN TEXT Section: **Selected Basic Physiology**. Pages 8 and 9, line 181-189

REVIEWER COMMENT 7

- Line 196: basic information on the mineralocorticoid receptor should be grouped here, especially the basic information on its affinity for both glucocorticoids and mineralocorticoids

RESPONSE:

We thank the reviewer for this comment. The initial rationale for discussing "**Challenges to the concept of adrenal functional zonation"** before the section on "**Regulation of aldosterone secretion, the mineralocorticoid receptor and mineralocorticoid activity"** was to first highlight the important histological concepts to consider at the site of steroid biosynthesis. This appeared to flow better. As a result of the reviewer feedback we have moved the section on **"Challenges to the concept of adrenal functional zonation"** to the beginning of the section on **"Selected basic physiology"**. As a result, information on the mineralocorticoid receptor, which includes the basic information on its affinity for both glucocorticoids and mineralocorticoids, now follows the section titled **"The stress response and steroid biosynthesis"** as suggested by the reviewer.

CHANGES IN TEXT Section: **Selected Basic Physiology**. Page 7, line 151 -169

REVIEWER COMMENT 8

- Table 3: Loss of negative feedback demonstrated for CIRCI: Multiple studies have demonstrated that corticotropin is reduced in critical illness, in accordance with increased feedback of cortisol on the level of the pituitary.

RESPONSE We thank the reviewer for this comment. We have amended Table 3 to reflect this.

CHANGES IN TEXT: Table File- Table 3 - in the revised manuscript. Revised text in red

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REVIEWER B

REVIEWER COMMENT 1

Gladness Dakalo Nethathe and colleagues wrote this comprehensive review on Critical illness-related mineralocorticoid insufficiency (CIRMI) a new term for described hyperrenemic hypoaldosteronism in critically ill patients. Overall, this review is well written and pleasant to read. The work well organized and comprehensively described with appropriate and adequate references.

My comments below are meant to improve this review.

Minor revisions:

• In order to support the statement concerning the variability of measurement of the cortisol and the corticotropin stimulation test in septic shock patient (section "Challenges with the confirmation of deficiency states" p17), the authors could add the following reference: "A single adrenocorticotropic hormone stimulation test does not reveal adrenal insufficiency in septic shock" (PMID: 16301260).

RESPONSE Thank you for this comment. We have added the statement as suggested. The section now reads as follows:

Challenges with the confirmation of deficiency states

Challenges with the diagnosis of CIRCI, based on the administration of synthetic corticotrophin, have been detailed elsewhere.(2,5) Briefly, as baseline total plasma cortisol levels are often variable in critical illness, currently accepted basal and stimulated cortisol levels, which were developed in healthy, non-stressed subjects pose difficulties.(105) Thus a single adrenocorticotropic hormone stimulation test does not reveal adrenal insufficiency in septic shock.(106)

CHANGES IN TEXT: Page 18 line 402-407

REVIEWER COMMENT 2

• In section "A comparison of therapeutic interventions", for more clarity, the authors should make a comparative table of the large RCTs (Annane et al, 2002, JAMA / Sprung et al, 2008, NEJM / Venkatesh et al, 2018, NEJM, Annane, 2018, NEJM) on hydrocortisone in septic shock.

RESPONSE Thank you for this comment. We have added the table as suggested and included the following text in the manuscript:

A comparison of large randomised controlled trials of hydrocortisone therapy in septic shock is presented in **Table 4.**

CHANGES IN TEXT Page 21 line 488-489 and Table 4 in the supplementary Table file Page line 600-602

REVIEWER COMMENT 3

• For less confusion, name the study by Annane and colleagues (p20-l443), the Ger-inf-05 trial.

RESPONSE We thank the Reviewer for this comment. This has been changed as advised by the reviewer as follows:

Section: **A comparison of therapeutic interventions.** This now reads as:

Four large studies on the use of corticosteroids for the reversal of septic shock reported conflicting results. In the Ger-Inf-05 trial ~~a study by Annane and colleagues~~, hydrocortisone therapy in patients with septic shock and adrenal insufficiency was associated with improved survival.(96)

CHANGES IN TEXT Section: **A comparison of therapeutic interventions.** Page 20 line 466-468

REVIEWER COMMENT 4

• P19-l439: Please, modified the sentence because there are some evidence on no beneficial effects of adjunctive corticosteroid supplementation in severe sepsis (HYPRESS trial, PMID: 27695824).

RESPONSE We thank the Reviewer for this comment and have made the suggested change as follows:

Corticosteroids may not be beneficial or may lead to a small reduction in mortality with a possible increase in the risk of hyperglycemia and neuromuscular weakness.(12,75,130,132,133)

CHANGES IN TEXT Page 20 line 463-465

REVIEWER COMMENT 5

• In the end of the section: "A comparison of therapeutic interventions", the authors should add some discussion concerning fludrocortisone replacement in critical ill patients:

o The only two trials (Annane et al, 2002, JAMA and Annane et al, 2018, NEJM) showing a decrease in mortality with steroid replacement therapy in septic shock included hydrocortisone and fludrocortisone in therapeutic group.

o In COIITSS (PMID: 20103758), 2 x 2 factorial, randomized trial, a secondary objective assessed the benefit of fludrocortisone in septic shock patients who received hydrocortisone, there was a –3% absolute difference in hospital mortality rates in patients treated with fludrocortisone plus hydrocortisone. This result was not statistically significant but this study was not adequately powered to detect a relevant treatment effect.

o The sentence about the FluDRes trial (p14-l302) could be moved to this part,

o The authors could be discussed angiotensin II therapy in vasodiltory shock (PMID: 28528561 / 32609011)

RESPONSE We thank the Reviewer for these comments as well as the suggestion to add a discussion on the role of Angiotensin II therapy for vasodilatory shock.

We have revised this section and added further text as follows:

The role of fludrocortisone, remains unclear. The only two trials demonstrating a decrease in mortality with steroid replacement therapy in septic shock included hydrocortisone in combination with fludrocortisone in the therapeutic group.(12,96) In *COIITSS*, a 2 x 2 factorial, randomized trial, a secondary objective assessed the benefit of fludrocortisone in septic shock patients who received hydrocortisone.(134) Patients were randomly assigned to 1 of 4 groups: hydrocortisone with continuous intravenous insulin infusion, hydrocortisone in combination with fludrocortisone with continuous intravenous insulin infusion, hydrocortisone with conventional insulin therapy, or hydrocortisone in combination with fludrocortisone plus conventional insulin therapy.(134)

Hydrocortisone in combination with oral fludrocortisone did not result in a statistically significant improvement in in-hospital mortality, however there was a –3% absolute difference in hospital mortality rates in patients treated with hydrocortisone in combination with fludrocortisone.(134) Although this result was not statistically significant, the study was not adequately powered to detect a relevant treatment effect.(134)

The administration of fludrocortisone in septic shock in the Activated Protein C and Corticosteroids for Human Septic Shock trial, was demonstrated to have a mortality benefit at 90 days.(12,135) However, on the contrary, the use of renin-angiotensin-aldosterone system antagonism, namely angiotensin converting enzyme inhibitors or angiotensin II receptor blockers, in the setting of sepsis has yielded conflicting results.(90,136)

Fludrocortisone has~~, in previous studies~~ been administered concurrently with high doses of hydrocortisone, doses purportedly high enough to have sufficient mineralocorticoid activity.(12,96)

However, effects of fludrocortisone that are not mediated through the mineralocorticoid receptor should be considered.(62,137,138) The implications of the differences in cellular downward signalling between the ligands, cortisol and fludrocortisone, acting on the mineralocorticoid receptor, require further clarification.(135) Importantly, the bioavailability of oral fludrocortisone in critical illness requires elucidation.(130,139,140)

The currently recruiting *Fludrocortisone Dose Response Relationship and Vascular Responsiveness in Septic Shock (*FluDRes) trial is a phase II, open label randomised controlled trial investigating the biological basis of vascular responsiveness in sepsis, as well as the pharmacokinetics and pharmacodynamics of fludrocortisone in septic shock.(141) Results of this trial will hopefully help address the role of combination therapy with hydrocortisone and fludrocortisone in the critically ill.

Angiotensin II has recently emerged as a non-catecholamine vasopressor agent in the management of septic shock.(13) In the *ATHOS-3 (Angiotensin II for the Treatment of High-Output Shock)* trial, patients with refractory high output shock were randomised to receive infusions of angiotensin II or placebo within a 48 hour study period. The administration of angiotensin II was associated with a positive vasopressor response to mean arterial pressure at 3 hours when compared to placebo. (114 (69.9%) vs. 37 (23.4%) [OR 7.95, p<0.001]). The measurement of renin levels was not part of the investigation in this trial. We hypothesize that in such populations hyperreninemic hypoaldosteronism may be concurrently present and we advocate for the assessment of hyperreninemic hypoaldosteronism in this population.

Indeed, Bellomo and colleagues recently used renin concentrations to identify patients that may benefit from angiotension II therapy.(142) Serum samples from patients enrolled in the *ATHOS-3* trial were assessed for renin, angiotensin I, and angiotensin II concentrations before the start of administration of angiotensin II or placebo and after 3 hours. In those with renin concentrations above the study population median, angiotensin II significantly reduced 28-day mortality to 28 of 55 (50.9%) patients compared with 51 of 73 patients (69.9%) of the placebo group (unstratified hazard ratio, 0.56; 95% confidence interval, 0.35 to 0.88; p= 0.012) (p= 0.048 for the interaction). A larger study assessing the response of aldosterone to angiotensin II along with renin measurements and with a patient centred outcome, such as duration of ICU stay or survival, would be of interest. Nonetheless, the finding, in this trial, that patients who had hyperreninemia were more likely to benefit from angiotensin II therapy highlights a population of catecholamine-resistant vasodilatory shock patients who have the potential to benefit from therapy modulating the renin-angiotensin-aldosterone system. As the maintenance of cardiovascular homeostasis is through multiple mechanisms, exploring the concept of synergy with regards to vasoactive medications in refractory vasodilatory shock, through the use of therapeutic approaches targeting both sympathetic tone and endocrine mechanisms, is a rational therapeutic approach.

CHANGES IN TEXT Page 22-24, line 491-547

REVIEWER C

REVIEWER COMMENT 1

The authors would like to define a new entity, critical illness related mineralocorticoid insufficiency. CIRMI.

They present a very thorough discussion of adrenocortical and mineralocortical pathophysiology in stress states.

RESPONSE Thank you for this comment

CHANGES IN TEXT Not applicable

REVIEWER COMMENT 2

They also describe false lowering of renin and aldosterone resistance. These discussions are distracting from the main issue and should be shortened.

RESPONSE Thank you for this comment. Aldosterone resistance is briefly mainly mentioned in the context of constructing the argument for comparing CIRMI with CIRCI. Hyperreninaemia could theoretically be a result of an element of aldosterone resistance. We would thus like to retain the limited discussion we have included on aldosterone resistance. The rationale being that CIRCI was understood as relative adrenal insufficiency, on the basis of cortisol levels and a corticotrophin response to ACTH. Reduced cortisol metabolism, as well glucocorticoid insensivity, have become accepted significant pathophysiological features of the syndrome.[3–5]

In the non-acute setting, aldosterone resistance has been described as a cause of hyperreninaemia.[6,7] However, in order to reduce the focus/emphasis on aldosterone resistance, as suggested by the Reviewer, we have removed the reference to aldosterone resistance in the conclusion. The following statement has been removed from the conclusion and appears as follows:

~~Comparative data on tissue aldosterone action in critical illness will add to current insights, as well as to the acquisition of diagnostic and therapeutic interventions.~~

CHANGES IN TEXT Page 26 line 590-591

REVIEWER COMMENT 3

While the discussion is thorough it does not satisfy the goal of defining this new entity. The narrative should center on the etiology of hyper-reninemic hypoaldosteronism and how it relates to CIRCI.

RESPONSE

We thank the Reviewer for this comment. We deliberated on two approaches to discussing this entity. One was to discuss it using evidence and rationale similar to

what has been presented for CIRCI. The second was to discuss our interpretation of hyperreninemic hypoaldosteronism as a form of mineralocorticoid dysfunction in its own capacity without the comparison to CIRCI per se. We find both methods to be valid provided that the evidence and observations presented for the hypothesis are critically evaluated and presented in a logical manner.

Indeed, we agree that the association with CIRCI is relevant. As such we found it most useful to discuss CIRMI in relation to this, particularly as there are currently accepted guidelines to defining CIRCI. More importantly, the two syndromes are linked due to the overlap and interaction of functions of many mediators involved in the stress response, as well as the proximity of zona glomerulosa cells to zona fasciculata cells in the adrenal (suggesting a common inflammation associated pathophysiological mechanism).[8]

As with CIRCI there would remain a number of areas that require further elucidation.

As more evidence becomes available- more specifically on the assessment of plasma renin activity and aldosterone in patients with shock, then the syndrome will become better refined.[9] Specifically with relation to the assessment of hyperreninemia in critical illness, we would refer to our previous publication which highlights the difficulties with assessment of renin in critical illness.[10] As such, definitive renin level criteria may prove to be an issue in critical illness much like definitive cortisol levels or the assessment of CIRCI using response to corticotrophin remains an issue.[11] Recent evidence supports the pragmatic approach of raising clinical suspicion when there are clinical findings of a shock state along with hyperreninemia.[12] Although the threshold criteria are based on limited evidence, we would suggest the consideration of adjunctive fludrocortisone therapy in the setting of an elevated plasma renin activity (PRA) in association with inappropriately low plasma aldosterone levels (ALDO). An ALDO/PRA ratio below 2 has been defined as inappropriately low in previous studies.[13]

However, much like the corticotrophin test, which is no longer routinely done before hydrocortisone administration, the routine assessment of ALDO/PRA ratios may be unnecessary. The response to aldosterone supplementation (haemodynamic and/or mortality improvement) would be the desired clinical effect. A randomised controlled trial like FluDress should address a number of these issues.[14]

With regards to the etiology of hyperreninemic hypoaldosteronism and its relation to CIRCI: The etiology of hyperreninemic hypoaldosteronism is hypothesised to be at the level of the adrenal. In the promotion of cortisol production during critical illness substrates are likely diverted away from aldosterone production. The hyperreninaemia is likely a result of relative hypoadrenalism. This likely occurs in a subset of patients with CIRCI.

We found it most conceptually useful to discuss CIRMI in relation to its counterpart CIRCI, hence the manner in which the proposed syndrome is presented. We improved

the text to clarify the etiological mechanism as follows:

Commonly associated with hypotension, hyperreninemic hypoaldosteronism is interpreted to represent a state of aldosterone deficiency.(11,91,95)

The etiology of hyperreninemic hypoaldosteronism is hypothesised to be adrenal dysfunction in a subset of patients with CIRCI.(11,91,95) As the zona glomerulosa does not appear to have all the enzymes required for aldosterone biosynthesis,(18) substrates are likely diverted away from aldosterone production in the promotion of cortisol production during critical illness, with the hyperreninemia likely a subsequent result of relative hypoadrenalism. Proposed etiological mechanisms are discussed in relation to CIRCI.

~~The administration of fludrocortisone in septic shock in the *Activated Protein C and Corticosteroids for Human Septic Shock* trial, was demonstrated to have a mortality benefit at 90 days.(11,95) However, on the contrary, the use of renin-angiotensin-aldosterone system *antagonism,* namely angiotensin converting enzyme inhibitors or angiotensin II receptor blockers, in the setting of sepsis has yielded conflicting results.(89,96) The currently recruiting *Fludrocortisone Dose Response Relationship and Vascular Responsiveness in Septic Shock (*FluDRes) trial is a phase II, open label randomised controlled trial investigating the biological basis of vascular responsiveness in sepsis, as well as the pharmacokinetics and pharmacodynamics of fludrocortisone in septic shock.(97) Results of this trial will hopefully help address the role of combination therapy with hydrocortisone and fludrocortisone in the critically ill.~~

~~Although an adequately powered study comparing fludrocortisone to a matched control group has yet to be completed,~~ Currently available data from adult and pediatric populations are contrasted and critically synthesised and an interpretation of the findings offered in **Table 1**.(96) Parallels with CIRCI are, however, apparent.

We then continue on to present further proposed etiological mechanisms in the section titled: **A comparison of critical illness-related corticosteroid insufficiency and hyperreninemic hypoaldosteronism**

CHANGES IN TEXT Page 14-15, line 313-335

REVIEWER COMMENT 4

First for those readers not in the ICU setting, CIRCI should be pragmatically defined as to levels of cortisol, ACTH stimulation and responses to therapy.

RESPONSE Thank you for this useful comment.

The diagnosis of CIRCI using laboratory parameters is controversial as discussed in the narrative review.[3] We had thus used the recommendations from a recent task force on the definition and management of CIRCI.[4]

We had defined it as:

Altered regulation of cortisol and aldosterone

Dysregulation of the hypothalamic-pituitary-adrenal axis associated with variable cortisol levels, altered cortisol metabolism, and tissue resistance to glucocorticoids are considered the three major constituents of CIRCI.[3,4,15]

We have now revised our text as follows:

A recent task team has described three major pathophysiological events in CIRCI; dysregulation of the hypothalamic-pituitary-axis, altered cortisol metabolism, and tissue corticosteroid resistance.(1) Although there is no single test that can reliably diagnose CIRCI, a delta cortisol (change in baseline cortisol at 60 min of < 9 µg/dL) after intravenous cosyntropin (250 µg) and a random plasma cortisol of < 10 µg/dL may be used.(5)

CHANGES IN TEXT Section: Background Page 5, line 103-107

REVIEWER COMMENT 5

The discussion should then describe hyper-reninemic hypoaldosteronism. There are only 5 articles over almost 40 years. The condition should be described as to the clinical settings (diagnoses, severity of illness) and the levels or renin and aldosterone and then describe the comparator groups and their renin and aldosterone levels. Several articles were 30 to 40 years ago, before our understanding of CIRCI and should be evaluated with more current knowledge

The authors should give details of the procedures to define the entity, and the comparator groups. What were the common etiologies, levels of illness by critical illness score and whether the low aldosterone levels were proportionate to low cortisol levels or low cortisol responses.

RESPONSE Thank you for this useful comment. We follow the introduction of CIRCI in the background section with a definition of hyperreninaemic hypoaldosteronism. The following sections in the manuscript describe the clinical setting of the condition and the definition of the entity:

1. Section heading: Background

Similar changes are evident regarding mineralocorticoid dysfunction in critical illness.(6,7) One such aldosterone dysfunction syndrome is selective hypoaldosteronism, which~~. Selective hypoaldosteronism~~ describes a sub-population of critically ill patients with an impaired adrenal aldosterone response to increased levels of renin, and is defined by the finding of hyperreninemia and hyperaldosteronism with

an aldosterone/plasma renin activity (ALDO/PRA) ratio below 2. Alternatively termed hyperreninemic hypoaldosteronism, selective hypoaldosteronism has been described in both hemodynamically unstable critically ill adult and pediatric patients with severe trauma and septic shock.(8-11)

CHANGES IN TEXT Not applicable- reference text in Section: **Background** Page 5-6 line 110-115

2. Section heading: Acute and chronic renin-angiotensin-aldosterone system activation and the role of aldosterone antagonism

Elevated plasma renin activity associated with relatively low aldosterone levels has been investigated as a marker of perfusion and prognosis.(8,91–93) Such a state of selective hypoaldosteronism, featuring a reduction in plasma aldosterone levels, despite rising corticotrophin and renin levels, suggests a dissociation between plasma renin and aldosterone.(88,94) Commonly associated with hypotension, hyperreninemic hypoaldosteronism is interpreted to represent a state of aldosterone deficiency.(11,91,95)

The etiology of hyperreninemic hypoaldosteronism is hypothesised to be adrenal dysfunction in a subset of patients with CIRCI.(11,91,95) As the zona glomerulosa does not appear to have all the enzymes required for aldosterone biosynthesis,(18) substrates are likely diverted away from aldosterone production in the promotion of cortisol production during critical illness, with the hyperreninemia likely a subsequent result of relative hypoadrenalism. Proposed etiological mechanisms are discussed in relation to CIRCI.

CHANGES IN TEXT Not applicable- reference text in **Section: Acute and chronic renin-angiotensin-aldosterone system activation and the role of aldosterone antagonism** Page 14, line 310-321

Table 1. Summary of published data from adult and pediatric populations on hyperreninemic hypoaldosteronism in critical illness provided in the supplement also provides a summary of the literature published on the entity.

With regards to levels of renin and aldosterone: An aldosterone/plasma renin activity (ALDO/PRA) ratio below 2 has been defined as inappropriately low in previous studies.[13] We have discussed, this as well as the methodological concerns, as follows:

As with CIRCI, challenges exist regarding the diagnosis of mineralocorticoid dysfunction in critical illness. Critical illness-associated hyperreninemic hypoaldosteronism is defined by a PA/PRA ratio <2, a definition based on values used

in non-critically ill patients with mineralocorticoid deficiency. This corresponds to the 98th percentile of the control population.(91) Low aldosterone levels, relative to plasma renin levels, have been demonstrated in pediatric meningococcal septic shock.(9) However, reference material and reporting units for both aldosterone and renin differ widely with internationally accepted standardised methodologies not yet in place.(94,114,115) Additionally, the diagnosis of hypoaldosteronism based on renin and aldosterone measurements using reference ranges obtained from non-critically ill populations poses a number of challenges.

Plasma renin activity measurement is based on indirect measurement of the combined effect of angiotensinogen and renin, indirect renin concentration measurement (involves the *ex-vivo* addition of angiotensinogen to the assay), and direct immunometric measurements.(116-118) Traditionally, plasma renin activity, which relies on the quantification of the cumulative generation of angiotensin I and not the direct quantification of renin, has been used.(118,119) Plasma renin activity relies on the available levels of angiotensinogen (renin substrate) and thus measures the combined effect of angiotensinogen levels and renin. Low angiotensinogen levels may thus lead to the underestimation of active renin concentrations, when plasma renin activity is measured.(120) Furthermore, when measured using direct assays, renin levels are shown to be suppressed by factors such as oestrogen or oestrogen therapy.(121)

Angiotensinogen has been shown to be elevated in adrenal insufficiency, by corticosteroid therapy, and in the setting of higher oestrogen levels.(117) Conditions common in critical illness, such as liver dysfunction and congestive heart failure are also associated with reduced angiotensinogen levels. The implications are that comparative use of data from studies where plasma renin activity was measured indirectly, as well as the implications that changes in the plasma concentration of angiotensinogen have on angiotensin II synthesis, highlight significant methodological concerns.(11,122) Further methodological concerns include the lack of validation of these tests for use in the critically ill, as well as implications of technical limitations in their performance (in the non-critical care setting plasma renin activity and serum aldosterone measurements should be performed after three hours in the upright or seated position as this increases renin and aldosterone release in normal individuals).(118) Currently, no reference ranges exist for plasma renin activity in critical illness.

Difficulties with the consistent demonstration of hypoaldosteronism in critical illness are compounded by the methods used to measure aldosterone.(11) Marked overestimation of aldosterone levels occurs in renal impairment with the use of homogenous immunoassays, likely as a result of antibody cross-reactivity with uncleared aldosterone metabolites.(123) The lack of standard reference ranges for aldosterone across populations, as well as variations in assay procedures among laboratories further compounds aldosterone level assessments.(124-126) High

performance liquid chromatography and tandem mass spectrometry (LC–MS/MS) is a more accurate method of determining aldosterone levels, however, due to cost implications and technical demands associated with LC–MS/MS systems, radio-immunoassays remain the methods of choice.(127,128) Currently, no published standardised LC–MS/MS reference method or standard reference materials are available.(123)

CHANGES IN TEXT Not applicable. Page 18-20 Line 411-449

REVIEWER COMMENT 6

If the entity is isolated aldosterone deficiency with retained cortisol responses then the authors should discuss possible etiologies i.e., renin inactivity, angiotensin 2 resistance, ACTH resistance, substrate limiting (adrenal substrate cholesterol) deficiency, enzyme inhibition (eg. natriuretic factor inhibition) or concomitant relative adrenal insufficiency.

RESPONSE

We thank the Reviewer for these useful comments.

There is a sub-group of critically ill patients with shock who have an impaired aldosterone response to renin suggestive of dissociation aldosterone and renin. Although a subgroup of the critically ill with hyperreninaemia whose mortality at 28 days was improved with angiotensinogen II infusion has been recently described, [12] in the first description of the syndrome a lack of an aldosterone response to angiotensin II or corticotrophin was described, suggesting a defect at the level of the zona glomerulosa.[16]

The aetiology is likely multifactorial. The proximity of the zona glomerulosa cells to the zona fasciculata cells is supportive of a unified inflammatory cause.[8] In addition substrate diversion to cortisol production is another potential cause. Furthermore cortisol is capable of down-regulating both mineralocorticoid and glucocorticoid receptors.[17]

As per our response to **REVIEWER COMMENT 3** The entity is not isolated aldosterone deficiency, hence the decision to discuss this concurrently with CIRCI. Possible causes are discussed in comparison to CIRCI in the section titled: **A comparison of critical illness-related corticosteroid insufficiency and hyperreninemic hypoaldosteronism**

CHANGES IN TEXT Not applicable Page 15-20 Line 339-399

REVIEWER COMMENT 8

From a practical point, they should give guidelines as to when we should measure

renin and aldosterone and for evidence of therapeutic manipulation.

RESPONSE

We thank the Reviewer for this comment and have modified our text to include the use of ALDO/PRA ratio of less than 2 in the assessment of CIRMI.

We have added the following text in the section titled: **Conclusion and future directions.**

An ALDO/PRA ratio below 2 has been defined as inappropriately low in previous studies and remains a criteria for definition until data from more recent studies becomes available.(8) We suggest the assessment of hyperreninemic hypoaldosteronism through the assessment of ALDO/PRA ratio with the consideration of co-administration of hydrocortisone with fludrocortisone in patients with septic shock. Unlike the non-acute setting cortisol levels are likely to be elevated in critical illness and they are of less diagnostic utility in the diagnosis of hyperreninemic hypoaldosteronism.

CHANGES IN TEXT Section: **Conclusion and future direction** Page 25, line 567-573

REVIEWER COMMENT 9

Is the entity a measure of severity of illness (such as low serum albumin)?

RESPONSE Yes. Again we thank the Reviewer for this comment.

We have added the following text in the manuscript to elaborate on the proposed etiological mechanism:

The etiology of hyperreninemic hypoaldosteronism is hypothesised to be adrenal dysfunction in a subset of patients with CIRCI.(11,91,95) As the zona glomerulosa does not appear to have all the enzymes required for aldosterone biosynthesis,(18) substrates are likely diverted away from aldosterone production in the promotion of cortisol production during critical illness, with the hyperreninemia likely a subsequent result of relative hypoadrenalism. Proposed etiological mechanisms are discussed in relation to CIRCI.

Of interest, in a recent study by Bellomo and colleagues, those with renin concentrations above the study population median, angiotensin II significantly reduced 28-day mortality to 28 of 55 (50.9%) patients compared with 51 of 73 patients (69.9%) of the placebo group (unstratified hazard ratio, 0.56; 95% confidence interval, 0.35 to 0.88; *P*= 0.012) (*P*= 0.048 for the interaction).(142) A larger study assessing the response of aldosterone to angiotensin II along with renin measurements

and with a patient centred outcome, such as duration of ICU stay or survival, would be of interest.

To the contrary, lack of response to angiotensin II and corticotrophin have been described as features of selective hypoaldosteronism. Further evidence will help clarify the role of angiotensin II therapy in patients with CIRMI.

CHANGES IN TEXT Page 14, line 316-321 and Page 23, line 532-547

REVIEWER COMMENT 10

Their comment that angiotensin is used in therapy to suggest that there is angiotensin deficiency or resistance would be analogous to saying that since norepinephrine is used in therapy there is norepinephrine deficiency.

RESPONSE We thank the reviewer for this comment and regret the alternative interpretation of the following comment in question from the Abstract section (Page 6 line 116 to 117):

The suggestion that angiotensin II is effective in treating vasodilatory shock further highlights the potential role of therapeutic approaches targeting the renin-angiotensin-aldosterone system in septic shock.(13)

The rationale for the above comment was to highlight various potential targets of the renin-angiotensin-aldosterone system (RAAS), not to suggest that there is angiotensin deficiency per se. Such potential effects would be the following: Apart from its effects on the RAAS, Angiotensin II remains a potent vasocontrictor. We understand the mechanisms behind the efffect and uses of Angiotensin II in vasodilatory shock as not being purely through its role as a direct vasoconstrictor. One should consider other mechanisms, such as, water reabsorption through potentiation of antidiuretic hormone, sodium retention via the synthesis of aldosterone, and through synergistic activity with catecholamines.[18]

However, and continuing with the adrenaline analogy, the role of adrenaline in vasodilatory shock from eg anaphylactic shock is through a number of mechanisms- not only as a vasocontrictor and inotrope due to its action on beta and alpha receptors but also as a mast cell stabiliser.[19] We agree with the Reviewer that none of these mechanisms suggest deficiency states but simply indicate the multiple targets of these agents in the treatment of shock.

We hope this clarifies things but are open to removing the statement if the Reviewer remains of the opinion that it would improve the abstract and minimise misinterpretations.

CHANGES IN TEXT Not applicable

REVIEWER COMMENT 11

The role of fludrocortisone should be discussed in relation to ref 91 and 92 regarding the role of concomitant glucocorticoids.

RESPONSE We thank the Reviewer for this comment. We have modified our text as advised. The discussion of the role of fludrocortisone is included as follows:

The role of fludrocortisone, remains unclear. The only two trials demonstrating a decrease in mortality with steroid replacement therapy in septic shock included hydrocortisone in combination with fludrocortisone in the therapeutic group.(12,96) In *COIITSS*, a 2 x 2 factorial, randomized trial, a secondary objective assessed the benefit of fludrocortisone in septic shock patients who received hydrocortisone.(134) Patients were randomly assigned to 1 of 4 groups: hydrocortisone with continuous intravenous insulin infusion, hydrocortisone in combination with fludrocortisone with continuous intravenous insulin infusion, hydrocortisone with conventional insulin therapy, or hydrocortisone in combination with fludrocortisone plus conventional insulin therapy.(134) Hydrocortisone in combination with oral fludrocortisone did not result in a statistically significant improvement in in-hospital mortality, however there was a –3% absolute difference in hospital mortality rates in patients treated with hydrocortisone in combination with fludrocortisone.(134) Although this result was not statistically significant, the study was not adequately powered to detect a relevant treatment effect.(134)

The administration of fludrocortisone in septic shock in the Activated Protein C and Corticosteroids for Human Septic Shock trial, was demonstrated to have a mortality benefit at 90 days.(12,135) However, on the contrary, the use of renin-angiotensin-aldosterone system antagonism, namely angiotensin converting enzyme inhibitors or angiotensin II receptor blockers, in the setting of sepsis has yielded conflicting results.(90,136)

Fludrocortisone has~~, in previous studies~~ been administered concurrently with high doses of hydrocortisone, doses purportedly high enough to have sufficient mineralocorticoid activity.(12,96)

However, effects of fludrocortisone that are not mediated through the mineralocorticoid receptor should be considered.(62,137,138) The implications of the differences in cellular downward signalling between the ligands, cortisol and fludrocortisone, acting on the mineralocorticoid receptor, require further clarification.(135) Importantly, the bioavailability of oral fludrocortisone in critical illness requires elucidation.(130,139,140)

The currently recruiting *Fludrocortisone Dose Response Relationship and Vascular Responsiveness in Septic Shock (*FluDRes) trial is a phase II, open label randomised controlled trial investigating the biological basis of vascular responsiveness in sepsis, as well as the pharmacokinetics and pharmacodynamics of fludrocortisone in septic

shock.(141) Results of this trial will hopefully help address the role of combination therapy with hydrocortisone and fludrocortisone in the critically ill.

CHANGES IN TEXT Section A comparison of therapeutic interventions **Page** 22-23, **line** 484-514

REVIEWER COMMENT 12

The concept is definitely worthwhile and I hope the authors would clarify the above clinical issues.

RESPONSE We sincerely thank the Reviewer for this encouraging comment as we hope we have satisfactorily addressed the Reviewers concerns.

CHANGES IN TEXT Not applicable

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