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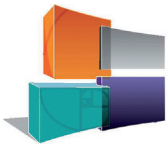

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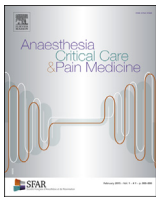

Editorial

CITRIS-ALI: How statistics were used to obfuscate the true findings

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The findings of the CITRIS-ALI study have recently been published [1]. A review of its abstract would lead the reader to conclude that intravenous vitamin C had no benefit in the treatment of acute respiratory distress syndrome (ARDS) induced by septic injury. Neither the significant mortality reduction nor the absence of side effects were mentioned in the abstract, and indeed downplayed in the paper itself. This was by editorial design to deceptively minimise the role that this potentially lifesaving, safe and cheap intervention may have in the management of patients with sepsis and sepsis induced ARDS. However, the science and the statistical evaluation of this publication are more complex and require further exploration.

Dr Fowler, the first author of the CITRIS-ALI study has been instrumental in a number of basic science and clinical studies that have advanced our understanding of the pathogenetic mechanism that vitamin C plays in sepsis, inflammation and ARDS [2]. Together with independent investigators across the globe, over 500 peer-reviewed experimental and clinical studies have been published, which have clearly demonstrated the biologic plausibility and mechanistic pathways for the treatment of sepsis and other inflammatory disorders with vitamin C alone and when combined with hydrocortisone and thiamine (see Fig. 1) [3,4]. The design of the CIRTIS-ALI study was largely influenced by a phase 1 study performed by Dr Fowler [5]. In this study, 24 patients with severe sepsis were randomised to receive an intravenous infusion of vitamin C (50 mg/kg/24 h, $n = 8$; 200 mg/kg/24 h, $n = 8$), or placebo ($n = 8$) every six hours for four days. The primary endpoints of this study were the safety and tolerability of the vitamin C infusions. No adverse safety events were observed in ascorbic acid-infused patients. Patients receiving ascorbic acid demonstrated a significant reduction in the SOFA score measured over the first 96 hours together with a significant reduction in the proinflammatory biomarkers C-reactive protein and procalcitonin. Prompted by these encouraging results and considering the effects of vitamin C on immune function, Dr Fowler and his group required funding to perform a larger randomised controlled trial (RCT). In 2011, The National Heart, Lung, and Blood Institute (NHLBI) offered grant funding for "Phase II Clinical Trials of Novel Therapies for Lung Diseases" (RFA-371 HL-12-022). Due to the failure of previous clinical trials to demonstrate a benefit in 28-day all-cause mortality as a primary endpoint, the NHLBI required that the primary endpoints focus on "quantifiable measures of organ function" (SOFA score) and biomarker analysis (CRP, procalcitonin, thrombomodulin) instead of mortality (a secondary endpoint). It was assumed that if primary outcomes were positive, this would translate into outcomes for secondary endpoints (including mortality). The unfortunate selection of this patient population (ARDS) and the non-patient-centred outcomes (as primary outcomes) as directed by the NHLBI grant explains the methodology and reporting of the CITRIS-ALI study. As reported in the study, the primary endpoint (change in SOFA score) was negative, yet there was a significant mortality benefit. While appearing difficult to reconcile these opposing findings, logical explanations exist.

The editor (and/or reviewers) went to great lengths to require the authors to emphasise that the mortality benefit was one of many secondary endpoints, which could have been significant purely by the play of chance. Furthermore, as the primary endpoints were negative, this must be a negative study. This is a very simple way of looking at the results. Mortality is an unambiguous and clearly defined endpoint that is of primary importance to patients, clinicians and all stakeholders. Furthermore, not all secondary endpoints are equal. Patients were fairly randomised to each group so the assertion that the play of chance led to this outcome appears unlikely. Secondly, due to a statistical aberration, known as "Neyman's bias" the assertion that the primary outcome was negative is likely flawed.

What is critical to appreciate is that mortality is important not just as an endpoint itself, but also because of its effect on the primary endpoint. The results showed a marked difference in mortality after 96 hours (5% in the vitamin C group *vs.* 23% in the control group) at which time the SOFA score was calculated. Since patients who died were not included in the primary endpoint, the change in SOFA score had eliminated the sickest 18% of patients from the control group (survivorship bias). These patients who died early would have had worsening organ failure and high SOFA scores. The true benefit of vitamin C for the primary endpoint (an organ failure score at 96 hours) might have been dramatically underestimated. This bias may explain the paradoxical finding of a higher survival at 96 hours with no significant change in their SOFA

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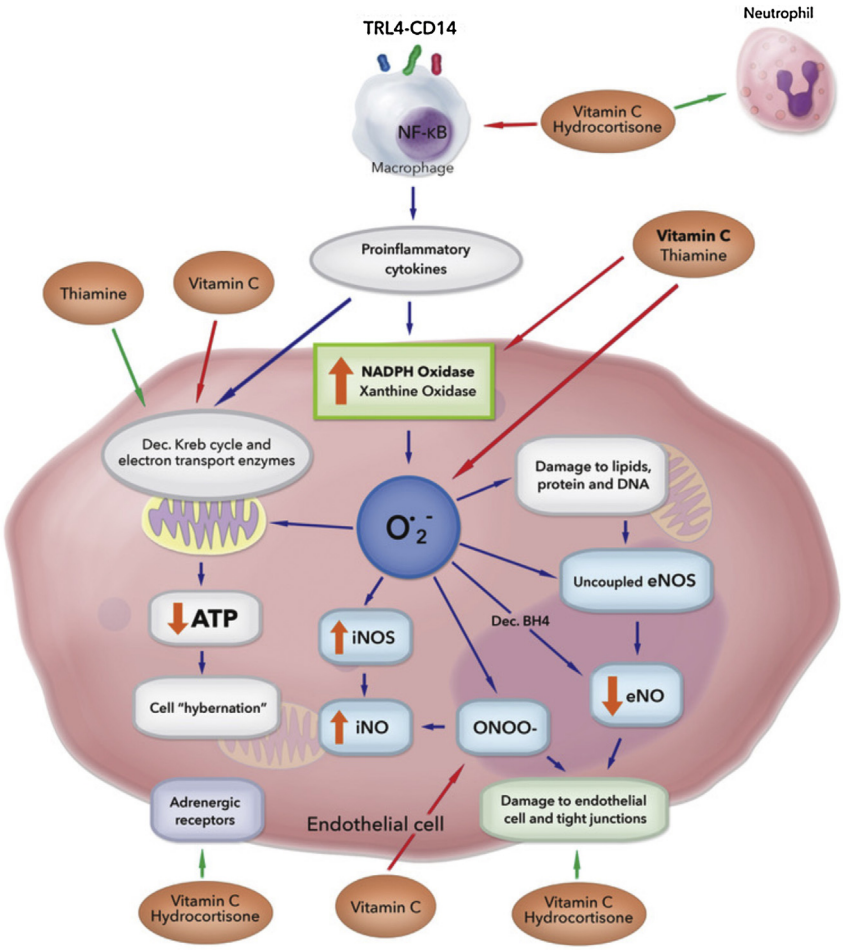

Fig. 1. Figure reproduced from Nutrients 2018; 10;1762 [1] under Open Access Policy. Multiple and overlapping effects of hydrocortisone, vitamin C, and thiamine in the setting of bacterial sepsis. Vitamin C and thiamine scavenge free radicals from superoxide ($O2^{\cdot-}$) and inhibit activation of xanthine oxidase and NADPH oxidase. Vitamin C protects the mitochondria from oxidative stress caused by increased leakage of electrons from the dysfunctional electron transport chain and recovers tetrahydrobiopterin (BH4) from dihydrobiopterin (BH2), restoring endothelial nitric oxide synthase (eNOS) activity and increasing eNO bioavailability. Vitamin C inhibits inducible NOS (iNOS) activation, preventing profuse iNO production and peroxynitrite (ONOO−) generation. Vitamin C scavenges ONOO−, preventing loosening of the tight junctions of the endothelium. Vitamin C and hydrocortisone decrease the activation of nuclear factor κB (NF-κB), thereby decreasing the release of proinflammatory mediators. They restore endothelial tight junctions and increase adrenergic receptor function. Thiamine increases the activity of pyruvate dehydrogenase and alpha-ketoglutarate dehydrogenase. These actions act in concert to restore cellular adenosine tri-phosphate (ATP) levels. ↑—increased levels/activity; ↓—decreased levels/activity.

scores. With such differential survival rates between the two groups, it is questionable to compare the SOFA scores at 96 hours only in the survivors to estimate how effective was vitamin C for these patients. The associated editorial *Journal of the American Medical Association* did not mention this paradoxical finding and emphasised only the failure of the primary endpoint to reach significance [6]. We propose to reanalyse the SOFA score taking the last one recorded before death during the 96-hour. As a consequence, we would not consider this to be a "negative" trial, but rather a faithfully executed study with optimistic results.

It is unfortunate that Dr Fowler's RCT had selected patients with sepsis and ARDS instead of those with early sepsis, as reported in the phase 1 study investigating the adjunctive role of intravenous vitamin C [5]. The CITRIS-ALI study evaluated the benefit of intravenous vitamin C in patients with established ARDS due to sepsis, which is a delayed complication (worsened by volume overload). Such a delay is illustrated in the CITRIS-ALI trial by the 32% of patients transferred from an outside hospital, suggesting a long course prior to enrollment. This time delay is fundamental; once organ failure(s) have developed they are less likely to be reversed [7]. Nevertheless, despite the fact that the CITRIS-ALI study investigated the use of intravenous vitamin C in ARDS, the authors were able to demonstrate a mortality benefit with this intervention.

The last debatable point concerns the selection of markers of inflammation. It is not certain that C-reactive protein and thrombomodulin were the best markers to assess inflammation. Their kinetics of plasma level changes and determinants do not fit well with the reported inflammatory modifications induced by Vitamin C. Therefore, it is difficult to conclude that Vitamin C did not change inflammatory intensity in the CITRIS-ALI study. It is also unfortunate that the authors chose too many secondary endpoints, as by a play of chance it is likely that a number of these endpoints would have been statistically significant. If the authors had limited the number to 4-6 clinically relevant endpoints, their interpretation would have been less controversial.

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None.

Disclosure of interest

The authors declare that they have no competing interest.

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