# CHAPTER 9.

## Summary and future perspectives

## Summary

In this thesis we assessed of adrenal dysfunction in the critically ill. The first part focuses on the pathophysiology and diagnosis of CIRCI. The second part discusses the use of corticosteroids in patients with a clinical suspicion of CIRCI and provides recommendations on how to diagnose and treat CIRCI in daily practice.

## Part I - Pathophysiology and diagnosis of CIRCI

**Adrenal sensitivity for endogenous and exogenous ACTH during critical illness**

Chapter 2 evaluates adrenal sensitivity for endogenous and exogenous ACTH in patients with a clinical suspicion of CIRCI. The adrenal sensitivity to endogenous ACTH was defined as the ratio between baseline cortisol and baseline ACTH. Sensitivity to exogenous ACTH was described as the increment of cortisol as a response to the ACTH test. The cortisol/ ACTH ratio has been previously used to diagnose primary adrenal insufficiency and to assess adrenal function after administration of etomidate[1-3]. However, to the best of our knowledge, this is the first report characterizing adrenal function during critical illness using the cortisol/ ACTH ratio. The hypothesis of this prospective cohort study was that diminished adrenal sensitivity to endogenous ACTH was associated with diminished responses to exogenous ACTH, irrespective of stage of the critical illness. To investigate this we included 59 patients with a clinical suspicion of CIRCI and measured total cortisol and ACTH, and performed an ACTH test followed by a second ACTH test after ≥ 7 days in acute phase survivors.

Subgroup analysis according to ACTH test response showed that patients with a diminished adrenal sensitivity to exogenous ACTH had higher baseline cortisol and ACTH levels, but a diminished adrenal sensitivity to endogenous ACTH (baseline cortisol/ACTH ratio) compared to patients with a normal response to ACTH. The cortisol/ACTH ratio did not change in time. Furthermore, patients with a low response to ACTH in time were more severely ill (higher APACHE II scores) and more often tended to have SIRS and sepsis.

In conclusion, our results confirmed our hypothesis. In patients with a clinical suspicion of CIRCI diminished adrenal sensitivity to endogenous ACTH was associated with diminished sensitivity to exogenous ACTH. In addition, several studies reported a cortisol-ACTH dissociation during the prolonged phase of critical illness suggestive of increased sensitivity to ACTH, ACTH independent cortisol production or decreased cortisol breakdown [4,5]. In contrast, in our study the cortisol/ACTH ratio did not change in time and our results therefore did not support the so-called cortisol-ACTH dissociation. The data further suggest a role of disease severity and culture positive sepsis as a risk factor for CIRCI.

**Steroidogenesis during CIRCI**

Mechanisms of adrenal dysfunction include impaired availability or cleaving of the substrate cholesterol and impaired activity of steroidogenic enzymes, limiting an adequate adrenal stress (ACTH) response. Also medication such as etomidate, a known inhibitor of 11β-hydroxylase promoting conversion of 11-deoxycortisol to cortisol, may result in a diminished response to ACTH [6-10]. In chapter 3 we present our study exploring steroidogenesis in patients with a clinical suspicion of CIRCI taking the use of etomidate into account. We therefore performed a prospective study including 62 critically ill septic and non-septic patients. All patients underwent ACTH testing and cortisol precursors (see figure 2, introduction) were measured while documenting previous use of etomidate.

We found that 21% of the patients received etomidate for intubation within 72 hours before blood sampling and ACTH testing. Forty two percent of patients had a diminished response to the ACTH test. Furthermore we demonstrated elevated baseline 11-deoxycortisol level, and depressed cortisol as well as cortisol/11-deoxycortisol, particularly in non-sepsis when etomidate was used. Baseline cortisol was higher in septic patients compared to non-septic patients. Furthermore, etomidate was associated with a lower cortisol response to ACTH. However, the frequency of a diminished cortisol response to ACTH was not significantly affected by the use of etomidate in septic and non-septic patients. The cortisol response to ACTH did not correlate with the baseline cortisol/11-deoxycortisol ratio neither in septic nor non-septic patients whether or not etomidate was used. Our data showed an association between diminished cortisol response to ACTH and low baseline cholesterol. Subgroup analysis according to ACTH test response and use of etomidate revealed no differences in 17-OH-progesterone, progesterone, corticosterone, corticosterone, aldosterone, DHEA and androstenedione levels, whereas etomidate use was associated with increased DHEAS levels. In conclusion, in agreement with Van der Voort et al. [11] a diminished response to ACTH testing was associated with lower cholesterol, suggesting substrate deficiency as a component of CIRCI. In addition, other rate limiting steps in the steroidogenesis were not found neither in septic nor non-septic patients with a diminished response to ACTH testing. Our data further suggested a limited contribution of etomidate to CIRCI, particularly in the septic patient.

**Adrenal haemorrhage as a potential mechanism of CIRCI ?**

In chapter 4 we describe a systematic review of the literature to summarize the reported functional consequences of adrenal haemorrhage. Our search yielded 62 original cases of adrenal haemorrhage in which the ultimate diagnosis was confirmed by imaging and quantitative results of measurements of cortisol with or without ACTH testing.

Analysis of patient characteristics showed that in most cases adrenal haemorrhage was bilateral (89%). Risk factors for adrenal haemorrhage were: surgery in 79%, anticoagulation in 39%, heparin-induced thrombocytopenia (HIT) in 27% and sepsis in 15% of the patients. All patients had a diminished response to the ACTH test. In 89% a very low response to ACTH was seen (<100 nmol/L). Adrenal haemorrhage was accompanied by a low baseline cortisol in 82% (276 nmol/L). Notably, 18% of the patients had relatively normal baseline cortisol levels suggesting that milder forms of adrenal dysfunction can be encountered in case of adrenal haemorrhage. In addition, in 67% of the patients with unilateral adrenal haemorrhage adrenal dysfunction was diagnosed. Follow up of adrenal function was not described in all cases. In 18% of the 62 case reports reversibility was reported, while irreversibility of adrenal dysfunction was reported in 21%.

Previous autopsy studies reported incidences of adrenal haemorrhage between 0.14 and 1.8% in general population [12]. By virtue of our study design it was impossible to estimate the incidence of adrenal haemorrhage, but underreporting is likely. Furthermore, due to publication bias, it is not known which proportion of patients with adrenal haemorrhage develops adrenal insufficiency. Nevertheless, our paper does represent the largest collection, we are aware of, of case series of adrenal haemorrhage documented by imaging and cortisol measurements.

In conclusion, adrenal haemorrhage can lead to adrenal dysfunction and can to some extent clinically resemble the inadequate cortisol availability in critical illness. Recognition may have therapeutic consequences such as interrupting heparin anticoagulation (in case of HIT) and administering corticosteroids. Biochemical testing and CT imaging in patients with a clinical suspicion for adrenal haemorrhage should be considered. Follow-up testing of adrenal function may be warranted because adrenal haemorrhage may even result in permanent damage to the adrenal gland.



**Use of total and free cortisol levels in the diagnosis of CIRCI**

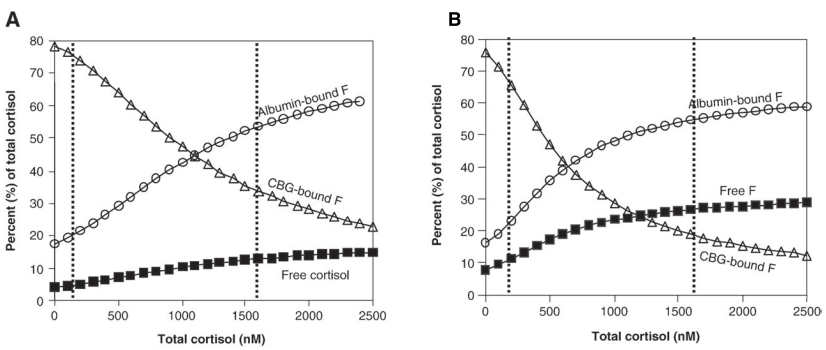
A decline in binding proteins during critical illness may lead to dissociation of total and free cortisol and thereby may confound the assessment of adrenal function testing when only total cortisol is used [13]. However, measurement of free cortisol is laborious, complex and not widely available. The aim of the study in chapter 5 was to investigate the additive value of free cortisol measurements compared to total cortisol in patients with a clinical suspicion of CIRCI. We therefore conducted a prospective study including 112 patients with a clinical suspicion of CIRCI. In all of these patients an ACTH test was performed and total and free cortisol (by equilibrium dialysis), CBG and albumin were measured. Our study revealed a strong correlation between free and total cortisol ($r = 0.77\text{-}0.79$, $P < 0.001$), although a low CBG was associated with higher free cortisol values in septic and non-septic patients. In

septic, hypoalbuminaemic patients, no dissociation was seen between total and free cortisol values (at baseline and 30 and 60 minutes after ACTH stimulation), and the increases in total cortisol in response to ACTH test predicted response of free cortisol regardless of low binding proteins. In agreement with Hamrahian, in non-septic patients total cortisol was lower in patients with hypoalbuminaemia than in those without, while free cortisol did not differ. In septic and non-septic patients the increment of cortisol upon ACTH stimulation depended less on binding proteins than cortisol values at baseline and 30 and 60 minutes after ACTH stimulation. Albumin poorly related to CBG in sepsis, but better in non-sepsis whereas both binding proteins were subnormal.

The lower total cortisol (rather than free cortisol) level in non-septic, hypoalbuminaemic patients with a relatively low CBG versus those with higher albumin levels may be clarified by the binding characteristics of CBG and albumin for cortisol. As demonstrated by Dorin et al. cortisol binding to CBG is saturable and characterized by a high affinity [14]. By contrast, cortisol binding to albumin has low affinity and is non-saturable (figure 1A). At increasing levels of cortisol, albumin bound cortisol and free cortisol levels will increase while CBG bound fraction declines. This leads to a predominance of albumin bound cortisol at high cortisol levels in case of combined CBG and albumin deficiency (figure 1B). Since hypoalbuminemia was not associated with lower CBG levels in septic patients, the latter may also help to explain that hypoalbuminemia in septic patients was not associated with lower total (rather than free) cortisol levels. Moreover, since sepsis may lead to damaged albumin molecules and thereby lower affinity of albumin for cortisol this may also explain why CBG rather than albumin affected free cortisol in sepsis [15].



**Figure 1.** Representation of relative concentrations of CBG-bound (open triangle), albumin-bound (open circle), and free cortisol (solid square) predicted by the cubic equilibrium solution. A. Under conditions of normal CBG and albumin concentrations. B. Under conditions of low CBG and albumin concentrations. Reproduced from Dorin R.I. et al. Validation of a simple method of estimating plasma free cortisol: role of cortisol binding to albumin. Clin. Biochem. 2009;42:64-71, with permission [9].



In conclusion, our study showed a close correlation between free and total cortisol, and their increases upon stimulation with ACTH. The additive value of free cortisol measurements in the diagnosis of CIRCI seems to be limited especially in septic patients if the total cortisol response to the ACTH test is used to diagnose CIRCI.

In chapter 6 we explored whether free cortisol can be reliably estimated using the Coolens and the adjusted Södergård equation. The Coolens equation estimates free cortisol value using measured total cortisol and taking CBG into account [16]. However, standard fixed values are used for the level and affinity of albumin to cortisol, unless adaptations are done. The Södergård equation was initially designed to estimate free testosterone levels by measuring total testosterone and taking the level and affinity of testosterone binding globulin and albumin into account [17]. Testosterone like cortisol, has a high affinity and saturable binding to testosterone binding globulin and a lower affinity, nonsaturable binding to albumin. De Ronde et al. adjusted the Södergård equation by using dissociation constants of CBG and albumin for cortisol [18]. We hypothesized that free cortisol calculated by the adjusted Södergård equation would better agree with measured free cortisol levels than that by the Coolens equation. In a prospective study we included 103 patients with a clinical suspicion of CIRCI and measured free and total cortisol, CBG and albumin. Free cortisol levels were calculated using both the Coolens and the adjusted Södergård equations.
Our data show a high bias and imprecision for estimating baseline free cortisol levels but also the free cortisol response upon ACTH testing in septic and non-septic patients for both the Coolens and the adjusted Södergård equations. The equations for estimating free cortisol levels may be affected by laboratory imprecision and changes in binding kinetics of cortisol to CBG and albumin during critical illness. The lack of agreement between calculated and measured free cortisol argue against the use of equations to estimate free cortisol levels in the critically ill.



## Part – II CIRCI and corticosteroids

The use of corticosteroids in patients with a clinical suspicion of CIRCI is still hotly debated. In chapter 7 we present a review in which we discuss the use of corticosteroids for its treatment during septic shock. The results of the CORTICUS trial [19] and other pivotal trials, such as the large trial by Annane [20] which did reveal survival benefit of the use of corticosteroids, are discussed. This review provides a background to interpret the results of these studies and to explain the differences in outcome between these studies. Furthermore, pros and cons of corticosteroid treatment are given. We conclude that the use of hydrocortisone to promote

shock reversal and survival in patients with fluid refractory, vasopressor-dependent septic shock, if initiated relatively early, can be beneficial. In addition, we suggest that it is likely that patients with the most severe disease are more likely to benefit from hydrocortisone treatment.

In chapter 8 we present a protocol concerning CIRCI accepted for publication in the book Critical Care Medicine edited by S.R. Villar. It gives the clinician a guide to recognize and treat patients with CIRCI. The pros and cons of the use of corticosteroids in patients with a suspicion of CIRCI are summarized in table 1 [21].

**Table 1**. Pros and cons of corticosteroid treatment in patients with a suspicion of CIRCI. ACTH: adrenocorticotrophic hormone; CIRCI: Critical Illness related Corticosteroid insufficiency; RAI: relative adrenal insufficiency. Reproduced from Molenaar N. et.al. Critical Illness-related corticosteroid insufficiency, Critical Care Medicine, Ed. S.R. Villar, in press with permission.



| PROS | CONS |
|---|---|
| • More rapid/frequent shock reversal<br>• Mortality benefit in some studies in high risk patients<br>• Effect most pronounced in patients with low cortisol increase upon ACTH, suggesting RAI/CIRCI<br>• Pharmacological doses useful in specific infections<br>• Useful when septic shock is associated with community-acquired pneumonia or acute respiratory distress syndrome<br>• May facilitate weaning from mechanical ventilation | • Diagnostic criteria for RAI/CIRCI non-uniform and controversial<br>• ACTH test results do not always predict effect of corticosteroids<br>• Mortality benefit controversial<br>• Elevated risk for hyperglycaemia, new infection and critical illness polyneuropathy. |

## Future perspectives

In this thesis we assessed adrenal dysfunction in the critically ill in order to gain insight into the pathophysiology, diagnosis and treatment of CIRCI. This thesis illustrates that the pathophysiology and diagnosis of CIRCI is extremely complex and that there remain many challenges to further unravel adrenal (dys)function during critical illness and its clinical consequences.

### Pathophysiology and diagnosis of CIRCI

The presented studies offer several overtures to further research. Questions concern the underlying mechanisms of CIRCI, optimal cortisol concentrations during critical illness and the role of corticosteroid resistance. We analysed adrenal dysfunction by novel and different methods, such as cortisol/ ACTH ratio, measuring free and total cortisol and exploring steroidogenesis in patients with a suspicion of CIRCI. We found that a diminished adrenal sensitivity to endogenous was associated with a diminished adrenal sensitivity to exogenous ACTH, suggesting CIRCI. Furthermore, our data did not support the ACTH-cortisol dissociation during prolonged critical illness as suggested by Vermes and Boonen [4,5]. Analysing steroidogenesis in patients with CIRCI suggested deficiency of the substrate cholesterol as a risk factor for CIRCI. In our systematic review we summarized adrenal dysfunction occurring in patients with adrenal haemorrhage. However, our study design, a systematic review of case reports, made it impossible to estimate the incidence of adrenal haemorrhage in the critically ill and the proportion of patients with adrenal haemorrhage resulting in adrenal dysfunction. A lot of work needs to be performed to further determine to what extent adrenal haemorrhage is an underlying mechanism of CIRCI. Especially the septic shock population would be of interest since the prevalence of CIRCI in these patients is estimated up to 60%. Possibly, adrenal haemorrhage as an underlying mechanism of CIRCI occurs more often than anticipated.

However, the pivotal question how much cortisol is enough for the severity of the illness of the critically ill patient still remains. The answer to this question is hard to establish. First, we found that total cortisol reliably reflect free cortisol levels and thereby measuring free cortisol is of limited additive value. Second, the answer to this question partially relies on whether plasma cortisol reliably reflects adrenal function and corticosteroid activity. Assessing adrenal dysfunction by combining diagnostic tools may be more accurate. For example, Dorin et al. sought to assess the adrenal function by calculating maximal estimating cortisol secretion rate and free cortisol half-life in patients with sepsis, septic shock and healthy volunteers as control subjects [22]. Another tool could be assessing corticosteroid activity by exploring the interstitium as demonstrated by Venkatesh [23]. The interstitial cortisol concentration is thought to represent the available cortisol pool which finally exerts its effect by binding to

the glucocorticoid receptor. However, none of these refinements accounts for corticosteroid resistance and this aspect of CIRCI was not investigated in our studies. Combining adrenal function testing with tools to evaluate corticosteroid resistance could to determine the optimal cortisol levels. Cohen et al. recently published the first prospective observational study measuring corticosteroid resistance in patients with septic shock and healthy controls [24]. Corticosteroid resistance was measured by an in vitro dexamethasone suppression test which depended on inhibition of cytokine production from lipopolysaccharide-stimulated leukocytes. Their results showed that patients with reduced corticosteroid sensitivity had a higher disease severity and possibly also mortality, the latter was not statistically significant. Nevertheless, the study did not have the power to draw robust conclusions. A larger prospective study is needed to elucidate the additive value of measuring corticosteroid resistance in patients with a suspicion of CIRCI.

**CIRCI and corticosteroids**



Despite continuing research, the use of corticosteroids in patients with CIRCI is still a matter of debate. Since 2008, when our review: "should we abandon corticosteroids during septic shock? No" was published, several trials resulted in more insight in CIRCI and the benefits and disadvantages of corticosteroids. So were the results of the multicentre randomized controlled COIITS study involving 509 adults with septic shock presented in 2010 [25]. Corticosteroids are associated with hyperglycaemia a complication that may affect patient's outcome while they are in the intensive care unit. The aim of the COIITS study was to test the efficacy of intensive insulin therapy in patients whose septic shock was treated with hydrocortisone and to assess, as secondary objective, the benefit of fludrocortisone. The study found that intensive insulin therapy did not improve in-hospital mortality compared with conventional insulin therapy among patients who were treated with hydrocortisone for septic shock. Furthermore, adding oral fludrocortisone did not significantly improve in-hospital mortality. In 2012 the surviving sepsis campaign adjusted their recommendation for corticosteroids in patients with sepsis to not treating septic shock patients when hemodynamic stability can be obtained by fluid resuscitation and vasopressor therapy. Only when this is not achievable they suggest hydrocortisone therapy [26]. The ongoing controversy concerning the use of corticosteroids in patients with sepsis and septic shock was accentuated by a systematic review with meta-analysis by Volbeda et al. This review demonstrated a lack of evidence to support or negate the use of high dose (>500 mg hydrocortisone daily) or low dose (≤ 500 mg hydrocortisone daily) corticosteroids in patients with SIRS, sepsis, severe sepsis and patients with septic shock [27]. In addition, the Cochrane collaboration updated the review of corticosteroids for treating sepsis in 2015. In total 33 randomized controlled trials were reviewed. In summary they suggested that corticosteroids may reduce 28-day mortality and

corticosteroids should be given in low dose and preferably in patients with septic shock, sepsis and ARDS, community acquired pneumonia or CIRCI as also suggested in our review [28]. Recently the results of the HYPRESS study were published [29]. This randomized controlled trial aimed to determine whether hydrocortisone may prevent development op septic shock in patients with sepsis. The authors concluded that hydrocortisone therapy did not reduce the risk of shock and therefore do not recommend the use of hydrocortisone treatment for adults with sepsis without signs of shock. Overall, although our review was written in 2008, recent studies are still in general agreement with our recommendations. The Cochrane review, most recent surviving sepsis campaign and the HYPRESS study underline our recommendation to start corticosteroids in patients with fluid refractory, vasopressor-dependent septic shock only and our statement that patients with the most severe disease are likely to benefit the most [28-30]. Recent insights may argue against our recommendation to treat patients with 200 to 300 mg of hydrocortisone daily. Because of the reduced cortisol breakdown and to minimize the risk of side effects of corticosteroids a lower dose of hydrocortisone could possibly be more appropriate, as also suggested by others [5,28-30].



Currently, the results of the APROCCHS trial are evaluated, a multicentre, randomized controlled trial initially designed to assess the benefit to risk ratio of activated protein C and corticosteroids, given alone or in combination in patients with septic shock [31]. Evaluation of activated protein C was terminated early because of withdrawal from the market. However, the trial continued the evaluation of corticosteroids for septic shock and replicated as much as possible the previously published study by Annane in 2002. Finally, 1241 patients were included and results are awaited. Another study, the important ADRENAL trial, is still running and aims to recruit 3800 critically ill patients [32]. It is designed to evaluate the use of corticosteroids during septic shock by comparing the effects of hydrocortisone and placebo on mortality, shock reversal, duration of mechanical ventilation and quality of life. In contrast to the APROCCHS trial, this study does not perform an ACTH test.

Another point of interest could be adding vitamin C and thiamine to the treatment with corticosteroids as suggested by Marik et al. [33]. Vitamin C is known for its antioxidant properties and may attenuate the exaggerated inflammatory response as seen during sepsis [33-34]. Due to its anti-oxidant effects, vitamin C may also decrease tissue glucocorticoid insensitivity. Adding vitamin C to corticosteroids may amplify the immune and haemodynamic modulating effects of corticosteroids resulting in a more balanced immune host response. A first before after study including 94 patients with sepsis and a procalcitonin $\geq$ 2 ng/ml found a remarkably lower mortality in the intervention group (8.5% vs. 40.4%) [35]. A large multicentre clinical trial is needed to test this hypothesis and to exclude possible adverse effects.

In conclusion, hopefully within the near future we are able to assess corticosteroid activity more accurately by taking patient characteristics, adrenal function testing and corticosteroid resistance into account. So, we will be better able to decide whom to treat with corticosteroids and whom not.

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