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Characterization of the Glucocorticoid Receptor in Children Undergoing Cardiac Surgery

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Abstract

Objective—Postoperative administration of corticosteroids is common practice for managing catecholamine refractory low cardiac output syndrome. Since corticosteroid activity is dependent on the glucocorticoid receptor (GCR), we sought to characterize GCR levels in children undergoing cardiac surgery and examined the association between GCR levels and cardiovascular dysfunction.

Design—Prospective observational cohort study.

Setting—Large, tertiary pediatric cardiac center.

Subjects—Children undergoing corrective or palliative cardiac surgery.

Interventions—None.

Measurements and Main Results—A prospective observational cohort study was conducted in 83 children with congenital heart disease. Total GCR levels were measured in the peripheral white blood cells using flow cytometry. In addition, blood samples were collected for total cortisol levels. The primary outcome studied was the time to being inotrope free (TIF).

An increase in GCR level from post-operative day (POD) 1 to POD 3 was associated with a longer TIF [HR=0.49 (0.29-0.81), p=0.01] in the univariate analysis. This association remained significant after adjusting for age, weight, cardiopulmonary bypass time, cross clamp time, RACHS-1 score, and post-op steroid use [HR=0.53 (0.29-0.99), p=0.05]. POD 3 GCR level

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showed a trend to have longer TIF [HR=0.66 (0.42-1.02), p=0.006]. The cortisol levels minimally increased during the study duration and did not correlate with GCR levels.

Conclusions—Increasing GCR levels in peripheral white blood cells of children undergoing cardiac surgery are associated with a longer TIF. Cortisol levels minimally increased during the study duration. These results suggest that exposure to high dose perioperative corticosteroids may suppress the hypothalamic-pituitary-adrenal axis leading to increase in GCR levels in response to a low cortisol environment. Further studies are required to better delineate the interplay between GCR levels, cortisol levels, corticosteroid exposure, and post-operative inotropic requirements.

Keywords

Steroids; Glucocorticoids; Glucocorticoid Receptor; Cardiac surgery; Low Cardiac Output Syndrome; Methylprednisolone; Adrenal insufficiency

INTRODUCTION

Cardiac surgery for congenital heart disease has evolved significantly in the last decades with complex repairs being possible with relatively lower mortality rates, however, complications continue to occur frequently. One of the prevalent complications is Low Cardiac Output Syndrome (LCOS)¹⁻³. LCOS is defined as decreased cardiac index in the early postoperative stage and is associated with higher vasoactive medication requirement, greater ventilator days, longer intensive care unit (ICU) stay and increased mortality⁴. Though the etiology of LCOS varies, the initial management uniformly includes intravenous (IV) administration of fluids and vasoactive medications. Interestingly, there is a subset of patients who appear to have a suboptimal response to vasoactive agents and some have speculated that such patients may have a degree of adrenal dysfunction and administration of corticosteroids may be beneficial. However, the precise etiology of adrenal dysfunction in this patient population it is not known. Studies involving pediatric septic shock patients suggest that there exists a group of patients who may be less responsive to corticosteroids based on repression of genes corresponding to the glucocorticoid receptor (GCR) signaling pathway^{5, 6}. Furthermore, preliminary data in critically ill children suggest that GCR expression varies widely, and that decreased GCR expression is associated with increased cardiovascular and other organ dysfunction⁷.

Glucocorticoids modify metabolic, immune and cardiovascular functions. The biological function of glucocorticoids is mediated through the activation of intracellular GCR. The glucocorticoid receptor is part of the superfamily of steroid/thyroid/retinoid acid receptor proteins that function as ligand-dependent transcription factors⁸⁻¹⁰. GCR is required for receptor translocation from the cytoplasm to the nucleus to affect gene expression^{11, 12}.

Until recently the only way to measure glucocorticoid activity in critically ill patients was through cosyntropin stimulation test, but our laboratory has developed a new technique for measuring GCR expression in white blood cells using flow cytometry⁷. We therefore, sought to characterize GCR expression in peripheral white blood cells in children undergoing cardiac surgery using this technique and to examine how GCR expression relates to cardiovascular dysfunction in this cohort.

METHODS

Patient and Data Collection

The study protocol was approved by Cincinnati Children's Hospital Medical Center (CCHMC) Institutional Review Board. Written informed consent was obtained from a parent or a legal guardian. This observational study was designed such that no added intervention will be given to the patients, instead patients receiving routine care as per CCHMC protocol will be followed prospectively between July 1, 2015 to January 31, 2016, and based on sample size calculation 83 subjects were enrolled. The patient enrollment was not disclosed to the care providers in the ICU during the study period.

Subjects requiring corrective or palliative cardiac surgery with cardiopulmonary bypass (CPB) and admitted to the Cardiac Intensive Care Unit (CICU) at CCHMC with an indwelling catheter (central venous catheter or an arterial catheter) were included. Those without informed consent, no central access available or underwent cardiac surgery without CPB were excluded.

Blood samples for GCR flow cytometry and serum cortisol levels were obtained after central access placement either in the operating room before surgery or on the morning of surgery prior to the perioperative administration of methylprednisolone in hospitalized patients. Subsequent blood samples were then drawn at 24 and 72 hours after surgery. Clinical and other laboratory data were recorded daily until hospital discharge or 28 days (which ever occurred later). All study subjects received a dose of 30 mg/kg of methylprednisolone as per CCHMC protocol at the time of CPB initiation to blunt the inflammatory response.

Laboratory procedure

GCR expression was measured with flow cytometry. In brief, after obtaining whole blood samples, the WBC count was determined and 1×10^6 cells are mixed with lysis buffer to remove red blood cells (Life Technologies, Carlsbad, CA). After centrifugation and wash steps, cells were incubated with human serum as a blocking step. After blocking and a series of washes, aliquots of cells were incubated with specific IgG against human leukocyte subset markers (Becton Dickinson, Valencia, CA). Each antibody was conjugated to a unique fluorophore for cell staining. After incubation with the cell surface antibodies and fixation in 2% paraformaldehyde, cells were permeabilized using a saponin-based buffer in order to measure surface (minimal) and intracellular (majority) GCR levels. At this point a second human serum blocking step was completed, and then incubated with either a mouse anti-GCR IgG diluted 1:50 (Thermo Scientific, Waltham, MA) or an isoform control mouse IgG1 diluted 1:10 (Abcam ab1264, Cambridge, UK). After incubation, cells were again fixed in 2% paraformaldehyde and stored in the dark until used for flow cytometry.

All flow cytometry procedures were conducted in the CCHMC flow cytometry core facility, counting 1×10^4 events for each antibody combination. Flow cytometry data was collected on BD Canto using FACSDiva v6.1.3 software (Becton Dickinson, Valencia, CA) and analyzed using Flowjo (Treestar, Inc). We controlled for non-specific staining by using an isoform control IgG and gating based on fluorescence minus one (FMO), meaning that all antibodies are included except for the anti-GCR IgG. Gating was established using FMO. We used MFI

for quantifying GCR expression levels, corrected for the respective FMO MFI. Cortisol levels were measured using the Access Immunoassay Systems with a potential cross-reactivity with prednisolone of 7%.

End points and outcome assessment

The primary outcome studied was the time to being inotrope free (TIF) defined as the time in days from surgery to the first day the subject was completely off inotropic agents. TIF is the equivalent to “inotropic support use” and “inotropic free days” commonly evaluated in cardiology, cardiac surgery and critical care studies in adults^{13, 14}. TIF or inotropic support use has been associated with outcomes in critically ill children, but has not been assessed in children undergoing cardiac surgery. This endpoint was chosen because mortality rates in congenital cardiac surgery are low outside of patients undergoing single ventricle palliation and based on sample size calculation it would have required a much larger sample to conduct a single-institution study.

The secondary outcome was LCOS and for this study LCOS for POD 1(post-op day1 arrival from OR to CICU until midnight), POD 2 and POD 3 was studied and was classified into five categories as *mild*: single vasoactive agent or arterial lactate < 2; *moderate*: two vasoactive agents or arterial lactate between 2 to 5; *severe*: three or more vasoactive agents or arterial lactate > 5; *mechanical support*: requirement of extracorporeal membrane oxygenation (ECMO) or ventricular assist devices (VAD); and finally, *death*. This endpoint was chosen because elevated lactate levels and more number of vasoactive agents with higher dosage have been associated with worse clinical outcomes^{1, 15, 16}.

Statistical analysis

Categorical variables were presented as frequency counts and percentages, and continuous variables as medians and interquartile range (IQR). The independent variables, GCR and cortisol were examined individually for each of the three study days (pre-op, POD 1, and POD 3). In addition, GCR was examined as an absolute change in levels between the three study days. Based on positive or negative change in GCR between the two study days, binary categorical variables as increase versus decrease in GCR levels between the study days were created to examine survival curves and Kaplan Meier curves were plotted to show the relationship between TIF, as time to event outcome, and change in GCR levels (increase versus decrease). Univariate and multivariable Cox proportional hazard models were developed to examine TIF in relation to GCR and cortisol. Multivariable models for TIF and GCR were adjusted for age, weight, CPB time, cross clamp time, RACHS-1 score, and post-op hydrocortisone use which were found to be significant factors in the univariate analysis and results were presented as hazard ratio (HR) and 95% confidence interval. Hazard ratios >1 were favorable in this study since shorter TIF was beneficial (as opposed to longer time being beneficial in usual survival models). The multivariable models for TIF and cortisol were adjusted for all covariates as for GCR, except post-op hydrocortisone use because interaction between cortisol and post-op hydrocortisone use was not-significant. For statistical analysis, LCOS was re-grouped into three categories by collapsing mechanical support and death into severe category because of low frequency counts. Association between TIF and LCOS scores as mild, moderate and severe for each individual post-

operative day (POD 1, POD 2 and POD 3) was examined using Cox model and hazard ratios were presented; hazard ratio >1 indicating shorter TIF and beneficial for this study. For secondary outcome as LCOS, proportional odds cumulative logit models accounting for repeated measures were developed and cumulative odds for severe and moderate LCOS versus mild was examined, and GCR and cortisol levels were the independent variables. All p values were calculated for 2-tailed test, with statistical significance set at $p = 0.05$.

RESULTS

Eighty-three consecutive patients were enrolled and examined. The demographic characteristics of the subjects are shown in supplemental table 1. The majority of the patients were infants (63%) with a median age 5.1 month (IQR: 0.5 - 43.6 month) and were males (63%). The most frequent diagnoses were conotruncal abnormalities (28%) and left sided obstructed lesions (24%). Only two patients required ECMO/VAD and 19% developed AKI. At POD1 13/83 (15.6%), POD2 18/83 (21.7%) and POD3 18/83 (21.7%) patients received postoperative hydrocortisone. The median TIF was 3 days (IQR: 1-5) and mortality was observed in 3 patients (3.6%). Change in GCR and cortisol levels over time is presented as supplemental Figure 1.

Figure 1 provides the Kaplan Meier curve demonstrating longer time to TIF with respect to increase versus decrease in GCR levels between POD 1 and POD 3 ($p=0.001$). In the univariate cox analysis, increase versus decrease in GCR levels from POD 1 to POD 3 also showed a significant association with longer TIF [HR=0.49 (0.29-0.81), $p=0.01$]. This association stayed significant in the multivariable analysis after adjusting for age, weight, CPB time, aortic cross clamp (ACC) time, RACHS-1 score, and post-op hydrocortisone use [HR=0.53 (0.29-0.99), $p=0.05$] (Table 1). Examining the relationship between TIF and GCR levels on each individual study day, a trend for longer TIF was observed for only POD 3 when adjusted for covariates [HR=0.66 (0.42-1.02), $p=0.006$] (Table 1).

Associating LCOS scores for each post-operative day with TIF, longer TIF was noticed with severe LCOS versus mild LCOS scores for all three post-operative days in univariate analyses. In the multivariable analyses, longer TIF was noticed with severe versus mild LCOS scores for POD3 [HR=0.15 (0.05-0.47), $p=0.001$] and for moderate versus mild LCOS [HR=0.28 (0.10 - 0.76), $p=0.01$] for POD3. For POD1 and POD2 severe versus mild LCOS scores trended to be associated with longer TIF (Table 2). Then examining LCOS as secondary outcome in relation to GCR levels as repeated measure (Table 3), the cumulative odds ratio of having severe and moderate LCOS versus mild LCOS were found to be less than 1 in both the univariate [OR=0.56(0.33-0.97), $p=0.04$] and multivariable models [OR=0.60 (0.37 - 0.97), $p=0.04$], indicating higher GCR levels tends to lower the LCOS severity.

The total cortisol level determination was found to have a wide range of distribution (Supplemental table 1). The median cortisol level increased from 12.4 $\mu\text{g/dl}$ pre-op to 14.4 $\mu\text{g/dl}$ on POD 1 and 20.6 $\mu\text{g/dl}$ on POD 3. Comparing TIF to cortisol on each study day, in both univariate and multivariable analyses higher cortisol levels were associated with longer TIF (Table 1). Comparing LCOS categories to cortisol, higher cortisol levels on POD 1 were

significantly associated with higher LCOS categories (Table 3). A trend was seen for negative correlation between GCR and cortisol levels pre-op and no significant correlation was identified between GCR levels and cortisol levels on POD 1 and POD 3 (Figure 2).

DISCUSSION

In this study, we characterized the GCR levels in white blood cells of children undergoing cardiac surgery requiring CPB and present their associations with clinical outcomes. The rationale for this study was the fact that cortisol levels have demonstrated low correlation with clinical outcomes in this patient population.

Our findings demonstrated a continuous increase in GCR levels throughout the first 72 hours following cardiac surgery. The increase in GCR level on POD 3 and increased levels between POD 1 and POD 3 were associated with longer TIF. Although the characterization of GCR in children undergoing cardiac surgery has not been documented until now, our findings were somewhat unexpected because studies in critically ill children and adults have demonstrated a decrease in GCR levels to be associated with more cardiovascular dysfunction and other organ failure¹⁷⁻¹⁹. The divergence of our findings from previous studies in non-cardiac patients may be explained by perioperative corticosteroids administration for CPB in children undergoing cardiac surgery and suggests an intricate interdependence between GCR levels and steroid exposure.

GCR regulation is a complex process and is influenced by several factors present during cardiac surgery in children. For instance, the duration of the exposure to corticosteroid has been shown to modify GCR levels²⁰. The majority of animal and human studies have demonstrated that a brief corticosteroid exposure causes decrease levels of GCR²¹⁻²⁴, however, other studies have demonstrated that continuous presence of corticosteroids leads to an increase in GCR levels^{20, 25, 26}. There is a possibility that hormone-level specific modulation could be a mechanism for cells to adapt to changes in the hormonal environment, though this mechanism has not been thoroughly studied. In addition, target organs modify GCR levels during specific situations such as infection, cancer or surgery. Sun et al. demonstrated increased GCR levels by studying protein and mRNA assessment in skeletal muscle of a rat model of sepsis and demonstrated that increased GCR levels augmented hormone binding activity during the catabolic response in sepsis²⁷. Al-Mohaisen et al. also demonstrated increased GCR levels in hepatic cells of rats exposed to acute and repeated immobilization stress and suggested that the increase in GCR levels may be an adaptive response for survival during stress²⁸. Thus, the interdependence between GCR levels, corticosteroid dose and duration of exposure may be different depending on the cell types and tissues under diverse stress conditions.

In terms of the secondary outcome, we identified that higher GCR levels in general were associated with lower LCOS categories during the first 72 hours postoperatively. However, the continuous increase in GCR levels were associated with longer TIF. Although a precise biological explanation for these findings is not available, other molecular mechanisms not analyzed during this study may participate in the interaction between cardiac function and the hypothalamic-pituitary-adrenal axis (HPA). For instance, the neuregulin-1 (NRG1) and

the erythroblastic leukemia viral oncogene homolog (ErbB), play a critical role in the regulation of the HPA axis activity and neurocardiac response to pathological stress²⁹. Specifically, the NRG1/ErbB pathway activation response can be biphasic, characterized by an initial adaptation phase that may protect the myocardium, followed by a maladaptive phase leading to disease progression^{30, 31}.

We also measured total cortisol levels in all subjects. Based on current definitions of adrenal insufficiency, the cortisol levels in general were suppressed and minimally increased following cardiac surgery. It has been shown that high dose perioperative corticosteroid treatment for CPB is associated with suppressed serum cortisol levels³². However, suppressed cortisol levels are particularly important in our study since all subjects received one dose of perioperative corticosteroids to blunt the stress response induced by CPB. Hence, our results may suggest that exposure to high dose perioperative corticosteroids suppresses the HPA axis and increases GCR levels as a result of a low cortisol environment. We also identified that higher cortisol levels were associated with longer TIF and higher LCOS scores. Although methylprednisolone is a selective GCR agonist, exposure to high dose corticosteroids and the endogenous glucocorticoid production may have activated both mineralocorticoid receptor (MR) as well as GCR³³. In fact, MR binds corticosteroids with comparable affinity, in contrast to GCR which binds glucocorticoids with higher affinity than aldosterone. Ultimately, excessive activation of the MR may contribute to cardiac dysfunction and has been associated with increased incidence of heart failure^{34,35}. Further studies are required to better delineate the interplay among corticosteroid exposure, cortisol and MR levels.

The primary limitations of the study include that white blood cells were used to quantify GCR levels under the assumption that they will be reflective of GCR levels in other tissues. The optimal approach would have been to quantify tissue GCR levels, but our study was designed to quantify GCR levels using a least invasive technique. The presence of GCR in cardiac cells has been determined^{20,23}, but whether GCR levels in white blood cells correlates well with GCR levels at other end organ level needs further investigation. The increase GCR levels after corticosteroid exposure may be tissue dependent as demonstrated in human leukemic T-cell line, myeloma cells and fibroblast from keloid tissue³⁶⁻³⁸. It is also important to indicate that the LCOS categories used for our study were based on perceptions of CICU providers and have not been rigorously validated³⁹. Furthermore, the use of cardiovascular agent exposure as a criterion for LCOS classification might not be as useful, particularly because some of the subjects in our study returned to the CICU on prophylactic vasoactive agents. Similarly, the duration of inotropic support may be an arbitrary time and dependent on the clinical and surgical staff caring for a patient. We attempted to minimize observer bias by blinding the clinical and surgical staff from the study. This study was powered to assess GCR performance in a heterogeneous population of children undergoing cardiac surgery and not for specific surgical repairs or palliations. The administration of glucocorticoids at the time of CPB initiation is considered standard of care at our institution, therefore, GCR levels were not determined on patients who did not receive them. Lastly, this is the first study examining association between TIF and GCR levels, and because TIF has not been standardized the results of this study may not be generalizable.

In conclusion, analysis of peripheral white blood cells of children undergoing cardiac surgery demonstrated an increase in GCR levels. The increase in GCR levels on POD 3 and between POD 1 and POD 3 were associated with a longer TIF. Cortisol levels minimally increased during the first 72 hours after surgery with no significant correlation with GCR levels. These findings may imply that peripheral white blood cells of children undergoing cardiac surgery are less sensitive to the down-regulating effects of glucocorticoids and may predispose patients to the negative side-effects of high doses of glucocorticoid treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Kaplan-Meier curve demonstrating Time to Inotrope Free (TIF) in relation to GCR levels

Figure 2.
Scatter plot presenting correlation between GCR and Cortisol levels

Table 1

Results of Cox-regression analyses examining association between Time to Inotrope Free (TIF), GCR and cortisol levels

GCR Expression and Cortisol analysis	Univariate models		Multivariable models	
	HR [LCL - UCL]	<i>p-value</i>	HR [LCL - UCL] [¶]	<i>p-value</i>
GCR				
Pre-op	1.13 [0.82 - 1.54]	0.45	1.19 [0.88 – 1.62]	0.26
POD 1	1.10 [0.88 - 1.38]	0.40	0.92 [0.69 – 1.22]	0.55
POD 3	0.79 [0.56 - 1.11]	0.17	0.66 [0.42 – 1.02]	0.06
Absolute change (Pre-op to POD 1)	1.03 [0.81 - 1.33]	0.78	0.86 [0.65 – 1.15]	0.32
Absolute change (POD 1 to POD 3)	0.82 [0.68 - 0.99]	0.04 *	0.88 [0.71 – 1.11]	0.30
Absolute change (Pre-op to POD 3)	0.82 [0.64 - 1.04]	0.11	0.75 [0.58 – 0.95]	0.02 *
Increase vs. decrease (Pre-op to POD 1)	0.91 [0.57 - 1.45]	0.70	0.67 [0.39 – 1.16]	0.15
Increase vs. decrease (POD 1 to POD 3)	0.49 [0.29 - 0.81]	0.01 *	0.53 [0.29 – 0.99]	0.05 *
Increase vs. decrease (Pre-op to POD 3)	0.85 [0.51 - 1.42]	0.53	0.76 [0.43 – 1.35]	0.35
	HR [LCL – UCL]	<i>p-value</i>	HR [LCL – UCL] [€]	<i>p-value</i>
Cortisol				
Pre-op	1.00 [0.76 – 1.30]	0.99	1.00 [0.74 – 1.36]	0.99
POD 1	0.82 [0.69 – 0.97]	0.02 *	0.90 [0.76 – 1.07]	0.23
POD 3	0.86 [0.72 – 1.03]	0.11	0.80 [0.64 – 0.99]	0.04 *

NOTE: All independent variables are continuous in nature except for increase versus decrease in GCR which were derived based on positive or negative change in GCR levels between study days. Shorter TIF was beneficial in this study (as opposed to longer time being good in usual survival models) therefore, hazard ratio >1 is favorable for this study. Hazard ratio <1 indicates longer TIF in relation to increase in GCR or Cortisol levels for each study day, or in relation to absolute change or increase versus decrease in GCR between study days.

[¶]Adjusted for age, weight, CPB time, ACC time, RACHS-I score, and post-op hydrocortisone use;

[€]Adjusted for all covariates as for GCR except post-op hydrocortisone use; HR, hazard ratio; LCL, lower confidence limit; UCL, upper confidence limit; POD 1, post-op day1; POD 3, post-op day3.

*
p 0.05

Table 2

Results of Cox-regression analyses examining association between Time to Inotrope Free (TIF) and LCOS scores for individual post-operative days

	Univariate model		Multivariable model ^{f/}	
	HR [LCL - UCL]	p-value	HR [LCL - UCL]	p-value
LCOS scores POD 1				
Severe	0.37 [0.19 - 0.71]	0.003 *	0.48 [0.21 - 1.07]	0.07
Moderate	0.68 [0.35 - 1.30]	0.24	0.75 [0.35 - 1.63]	0.47
Mild (ref)	1		1	
LCOS scores POD 2				
Severe	0.25 [0.13 - 0.50]	<0.0001 *	0.41 [0.16 - 1.05]	0.06
Moderate	0.60 [0.31 - 1.18]	0.14	0.83 [0.36 - 1.91]	0.66
Mild (ref)	1		1	
LCOS scores POD 3				
Severe	0.13 [0.05 - 0.32]	<0.0001 *	0.15 [0.05 - 0.47]	0.001 *
Moderate	0.28 [0.13 - 0.64]	0.002 *	0.28 [0.10 - 0.76]	0.01 *
Mild (ref)	1		1	

NOTE: Shorter TIF was beneficial in this study (as opposed to longer time being good in usual survival models) therefore, hazard ratio >1 is favorable for this study. Hazard ratio <1 indicates longer TIF in relation to severe versus mild, and moderate versus mild scores. POD, post-op day;

^{f/}Adjusted for age, weight, CPB time, ACC time, RACHS-I score, and post-op hydrocortisone use; HR, hazard ratio; LCL, lower confidence limit; UCL, upper confidence limit;

* p 0.05

Table 3

Results of Proportional Odds models examining association between LCOS scores, GCR and cortisol levels

Variables	Univariate Models		Multivariable Models [¶] €	
	OR [LCL - UCL]	p-value	OR [LCL - UCL]	p-value
<i>A) Examining all three study days longitudinally</i>				
GCR	0.56 [0.33 – 0.97]	0.04 [*]	0.60 [0.37 – 0.97]	0.04 [*]
Cortisol	1.28 [0.91 – 1.80]	0.15	1.23 [0.89 – 1.71]	0.20
<i>B) Stratified by each study day</i>				
Pre-op				
GCR	0.64 [0.36 – 1.16]	0.14	0.55 [0.29 – 1.06]	0.07
Cortisol	0.58 [0.33 – 1.02]	0.06	0.56 [0.29 – 1.10]	0.09
POD1				
GCR	0.63 [0.39 – 1.02]	0.06	0.64 [0.34 – 1.18]	0.15
Cortisol	1.93 [1.27 – 2.92]	0.002 [*]	1.83 [1.10 – 3.03]	0.02 [*]
POD3				
GCR	1.99 [0.79 – 5.04]	0.14	1.26 [0.38 – 4.18]	0.70
Cortisol	1.41 [0.84 – 2.35]	0.19	1.52 [0.79 – 2.93]	0.21

[¶]GCR models adjusted for age, weight, CPB time, ACC time, RACHS-I score, and post-op hydrocortisone use;

€Cortisol models adjusted for all covariates as for GCR except post-op hydrocortisone use; OR, odds ratio; LCL, lower confidence limit; UCL, upper confidence limit;

^{*} p 0.05. An odds ratio <1 indicates lower odds of having severe and moderate LCOS versus mild LCOS in relation to increase in GCR or Cortisol levels examined for all three study days (A) or by each study day (B) - Pre-op, POD1 and POD3.