Characterization of glucocorticoid receptor expression in neutrophils and monocytes of critically ill children

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Critical illness-related corticosteroid insufficiency (CIRCI):

Inadequate corticosteroid activity for the severity of the patient’s illness, generating an exaggerated and protracted pro-inflammatory response.

Marik, Crit Care Med 2008; 36:1937

Occurs in association with:

- Septic Shock (Annane et al 2002)
- Acute Respiratory Distress Syndrome (Meduri et al. 2007)
- Traumatic Brain Injury (Cohan et al 2005)
Glucocorticoid receptor (GCR)

Genome-wide expression profiling study

- 3 subclasses of children with septic shock
- 1 Subclass with repression of genes related to GCR signaling pathway
- Higher severity of illness and mortality

Wong, BMC Medicine 2009; 7:34
Glucocorticoid Receptor

Diffuses from circulation across the cell membrane

Binds to intracellular GCR

Cortisol-GCR complex: anti-inflammatory

Inhibits NF-KappaB’s transcription of inflammatory mediators

Barnes et al, NEJM 1997
Forms of CIRCI

Critical Illness Related Corticosteroid Insufficiency
"Relative adrenal insufficiency state"

- Decreased cortisol:
  - Circulation
  - Production
  - HPA axis

- Blood GCR

Annane 2002
Corticus 2008

Decreased tissue response to glucocorticoids

ACTH stimulation test
There may be a subset of critically ill patients who are relatively unresponsive to cortisol due to a decreased GCR expression and present with a peripheral resistance form of CIRCI.

In this preliminary study in PICU population we tested the hypothesis that GCR expression correlates with severity of disease and the need for cardiovascular support.
Methods

- Flow cytometry based analysis of GCR expression in neutrophils and monocytes isolated from whole blood using a human specific antibody (Glucocorticoid receptor monoclonal antibody, FITC conjugate 5E4 – Thermo scientific) and random cortisol level.

- FMO-GCR values were subtracted from cell-type specific mean fluorescence (MF) values and the resulting MF was analyzed for correlation with clinical data.
Flow cytometry analysis

FMO

Isotype

GCR
• Sample size: 25 critically ill children
• Demographics:
  • Median Age: 96 months (IQR 22.5-131)
  • Median PRISM III: 9 (IQR 2-12)
  • Median Organ Failure: 2 (IQR 1-3)
  • 11 received inotropes or vasoactive drugs (44% - Shock group)
  • 12 received steroids (48%, steroids group)
  • Mortality: 1 (4%)
Results: Monocytes GCR MF

Median 1044
IQR 818-1267
Min value 260
Max value 2795
**Results: Neutrophils GCR MF**

Median: 581

IQR: 434-781.5

Min value: 228

Max value: 3810
Main Results: Monocytes

PRISM III

Organ Failure

p=0.095

Lower 50% CD14

Median: 5

IQR (0-11)

Higher 50% CD14

Median: 11

IQR (5.5-13.5)

p=0.327

Lower 50% CD14

Median: 2

IQR (0-2.5)

Higher 50% CD14

Median: 2

IQR (1-3)
Main Results: Neutrophils

PRISM III

Lower 50% CD66b

Higher 50% CD66b

Organ Failure

Lower 50% CD66b

Higher 50% CD66b

p = 0.240

Median: 5

IQR (0.5-11.5)

p = 0.356

Median: 2

IQR (0-2.5)

Median: 10.5

IQR (3.25-13.5)

Median: 2

IQR (1-3)
Results: other specific findings

- Age
- Random cortisol level
- Need for inotropes or vasoactive drugs
- Steroid administration

Did not show correlation in both monocytes and neutrophils in this small study population.
Conclusions

• There is great individual variability of GCR expression in neutrophils and monocytes of critically ill children.

• There is a trend for a direct correlation between GCR expression and PRISM III values, suggesting that sicker individuals have a higher expression of glucocorticoid receptors in monocytes and neutrophils.

• We speculate that this is an adaptive mechanism for regulating the inflammatory response.

• This trend and the negative correlations found need to be confirmed with a larger patient sample.
Next steps

- GCR expression Septic Shock
- GCR expression in Lymphocytes
- Separate analysis of GCR subunits (Alpha and Beta)
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Antibodies

- **Pacific Blue** Mouse Anti-Human CD4 – BD Pharmigen
- **Alexa Fluor 700** Mouse Anti-Human CD8 – BD Pharmigen
- **Alexa Fluor 647** Mouse Anti-Human CD66b – BD Pharmigen
- **PE (Phycoerythrin)** Mouse Anti-Human CD14 – BD Pharmigen
- **Isotype:** Mouse IgG1 (HyblgG1) *(FITC)* - abcam
- **GCR:** anti-Glucocorticoid receptor *(FITC)* Mouse, clone: 5E4 MA1-81793 – Thermo scientific