Biomarkers and interventions for life-threatening infections

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Case: cerebral malaria
- 32 yr old tri-athlete \( \rightarrow \) climb Kilimanjaro
- T.O. \( \rightarrow \) fever \( \rightarrow \) Emergency \( \rightarrow \) Dx Pf malaria
\( \rightarrow \) Rx IV quinine
- Coma \( \rightarrow \) Dx: cerebral malaria (CM)
\( \rightarrow \) Develops multi-organ failure \( \rightarrow \) dies day 4
Two Problems:
Can’t recognize those \( \rightarrow \) adverse outcomes
Can’t seem to offer \( \rightarrow \) effective intervention
Fatality rates CM \( \rightarrow \) Art 30% vs 39% Quinine
Dondrop A. et al. Lancet 2005 SEAQUAMAT

Sub-Saharan Africa:
Same Problems \( \rightarrow \) Bigger scale
Can’t recognize those \( \rightarrow \) at risk of severe infection
- \( \sim \) Only 1-2% of children with malaria \( \rightarrow \) severe and potentially fatal malaria
- Clinic \( \rightarrow \) see 500+ children/day \( \rightarrow \) miss the 5 with at risk of fatal outcome \& misallocate health $$
- If it were possible to rapidly identify children at risk \( \rightarrow \) hospitalized and maximize the impact of limited health resources to save lives

Cerebral Malaria
Therapeutics to improve outcome?
“despite aggressive use of supportive therapy and effective antimalarials, the case fatality rate of cerebral malaria has not decreased in 50 years”
N White. Lancet

Antimicrobials are not enough:
- H5N1 can cause high fatality rates in young healthy individuals
- Intense inflammatory response central to pathogenesis of H5N1
- Similar story with SARS CoV, Sepsis and “SBI” such as invasive pneumococcal and meningococcal infections
“Its about the host”
Antimicrobials are not enough  
*Sir William Osler 1904*

“It appears that patients are dying not from their infections, but rather their reaction to them”

Severe Infections:

- The capacity to rapidly identify children at risk of severe malaria and the ability to apply novel interventions to improve survival would represent a transformative advance.

Severe Infections

Hypotheses:

- Define host responses that contribute to severe and fatal outcomes
- Identify “biomarkers” of those pathways
- Identify those at risk
- Identify inhibitors of those pathways
- Novel therapies for life-threatening disease

Cerebral and fatal malaria

What goes wrong?

- Parasites replication overwhelms host clearance
- Excessive inflammatory response
- Endothelial activation
- Malaria adhere in the brain
Background on PKD and malaria

Previous murine *in vivo* model *P. chabaudi*

Min-Oo G and al. Nature Genetics 2003

Mice deficient for Pyruvate Kinase
- Lower parasitemia
- Faster clearance
- Reduced mortality
- Homozygous mutation in the PK gene AcB55/AcB61

PKD is protective for Pc → mechanism of protection??

Conclusion

Common Mechanism of protection in Sickle cell, Thalassemia, G6PD, PKD, ABO:
- Enhanced phagocytosis of parasitized RBCs
- Can we pharmacologically turn on this uptake pathway?
  → lower parasite burden
  → decrease risk of severe and cerebral malaria

Conclusions

- challenges a classical tenet of malaria
- suggests that selective up-regulation of mΦ
  CD36 useful in treatment.

McGilvray et al. Blood 2000
Serghides and Kain. J Immunology 2001
Serghides and Kain. Lancet 2002
Serghides et al. Trends Parasitol 2003
Serghides and Kain. Lancet 2003
Patel et al. JID 2004
Ayi et al. Infect Immun 2005
Serghides and Kain. Infect Immun 2005
Zu et al. J Immunology 2006
Patel et al. J Immunology 2007
Increasing parasite clearance with Rosiglitazone

- CD36-expressing Monocyte/Macrophage
- CD36-binding PE
- PPARγ-RXR agonists e.g. Rosiglitazone

Increased CD36 expression
Increased CD36-mediated phagocytosis of PEs

Rosiglitazone protective even when administered up to 5 days post infection

Randomized double blind placebo controlled trial of Rosiglitazone as adjunctive therapy for P. falciparum malaria

Collaboration: Prof. Srivicha Kruddsood and Prof. Sornchai Looreesuwan, Mahidol

Primary outcome:
- 50% and 90% parasite clearance times (PCT)

Secondary Outcomes:
- Fever clearance (FCT)
- Safety and tolerability
- Biomarkers

Rosiglitazone improves parasite clearance times in patients with falciparum malaria

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rosiglitazone</th>
<th>Placebo</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parasite clearance time 50% (h)</td>
<td>19.0 (15.4)</td>
<td>24.6 (19.1)</td>
<td>0.029</td>
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<tr>
<td>Mean (SD)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Range</td>
<td>3 - 72</td>
<td>3 - 75</td>
<td></td>
</tr>
<tr>
<td>Parasite clearance time 90% (h)</td>
<td>30.9 (18.2)</td>
<td>40.4 (21.9)</td>
<td>0.0035</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>5 - 78</td>
<td>5 - 84</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: LFT, liver function tests; Cr, creatine.
Rosiglitazone reduces parasite burden in patients with malaria

Rosiglitazone improves fever resolution in patients with malaria

Rosiglitazone reduces levels of inflammatory biomarkers in patients with malaria

Summary

- To deaths due to Cerebral Malaria
- modify host response to infection
- class of cmpds
  - clearance
  - infection-induced inflammation
  - experimental cerebral malaria
- Safe and well tolerated in a RCT
  Went from basic hypothesis all the way to a RCT in ~5 years for ~$500K

Why the endothelium?

Kubes P. Nature Immunol 2009;9:364

- Largest organ linking all others
- >60 trillion cells and ~4000 m²
- Activation common pathway in critical illness (e.g. sepsis, VHF, DSS, HUS, TSS)
- Massive surface area & PRRs (TLR2,4,9) → essential surveillance organ
  → contributes to life-threatening response & progression to critical illness → MOF

Cserti C et al. 2006
Angiopoietins

- **Angiopoietin-1**
  - Binds the Tie-2 receptor
  - Mediates endothelium quiescence

- **Physiological Conditions:**
  - Ang-1 \(\uparrow\)
  - Ang-2 \(\downarrow\)

- **Angiopoietin-2**
  - Antagonistic factor
  - Blocks Ang-1/Tie2 binding
  - Role in neoplasia and angiogenesis

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### Biomarkers for cerebral malaria (CM)

- **AUROC curve** = 1.0 for ang1 and ang2/1
- Sensitivity and Specificity for cerebral malaria = 100%

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### Next Steps

- **Confirm in African children**
- **Establish causal role:**
  - Floxed Ang1 Balb/c: CM-R \(\rightarrow\) CM-S
  - Floxed Ang2 B6: CM-S \(\rightarrow\) CM-R
  - Ang2 KO B6 mice
- **Examine interventions:**
  - Inhalational NO (NO is a major regulator of Ang-2 release from endothelium)
  - AdenoAng1
  - MSCs expressing Ang1

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### CM in African Children

- **Collaborators:** Chandy John (U Minn) and Robert Opoka, Makerere University, Mulago Hospital, Kampa, Uganda
- **Serum samples from consecutive children with CM, UM or healthy controls**
  - Healthy controls (HC; N=28)
  - Uncomplicated Malaria (UM; N=67)
  - Cerebral Malaria WHO (CM; N=69)
- **Diagnosis of P. falciparum malaria by microscopy**
- No retinopathy but following cognitive function in survivors

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### Admission levels of Ang-1 and Ang-2 predict subsequent mortality

![Graph showing admission levels of Ang-1 and Ang-2](Lovegrove et al. PLoS 2009)
The Future of Diagnostics

What are Biomarkers and “Theranostics”?
- The next generation of diagnostics
  - Reliably detect and categorize pathogens
  - Determine severity of illness and facilitate management
- Desirable attributes of biomarker:
  - Improves clinical decision making
  - Has economic value (prevents unnecessary drug use or admission)
  - Has prognostic value
  - Enhances subject selection for research
- Collectively, “theranostics” are individualized molecular diagnostic/prognostic biomarkers
  - They replace subjective elements of clinical decision making, permitting evidence-based, individualized therapeutics

High Impact Diagnostics

Urdea et al. Nature 2006;S73

- Malaria (<1 h)<500 para/uL)
  Sen 90% and Spec 90% (point-of-care)
  \(\rightarrow 2.2M\) Dalys and \(~477\)million unnecessary treatment courses (~$1B USD/yr)

Take home points
- Host response is a critical determinant of survival
- Understanding host-pathogen interactions may identify
  - Biomarkers for diagnosis, prognosis and clinical decision making \(\rightarrow “theranostics”\)
  - New therapeutic targets
- Old drugs may have new tricks (fastest way to a new drug is to find a new indication for an old one!)