Preventing malaria-associated death and disability

Why outcomes matters

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The Problem

• Everyone gets infections but few become critically ill (~1:100-1000s)
  ➔ we have inadequate tools to rapidly recognize and triage them

• Once a severe infection (SM) occurs, there are high fatality rates despite ICU and antimicrobials

• Survivors ➔ brain injury ➔ We have no specific treatments to improve outcome
Barriers to Impact: LMIC

- ~50-80% of children die without engaging the formal health care sector → IMPACT requires a community-based solution
- We need an integrated approach to the febrile child NOT a pathogen-based one → etiology-based Dx alone (e.g. Pf RDT; 90% neg) does not inform critical decisions e.g. admit, PO or IV

Etiology is not enough

V D’Acremont et al. NEJM 2014
Beyond Malaria — Causes of Fever

- **FIRST:** assessment of etiology → misleading e.g. only 9% Pf+ → 50% of those had 2nd agent
- Multiple pathogens → ascribing causality?
- Colonization versus disease e.g. severe pneumonia detection of resp. virus: cases 60% vs 47% controls
- **SECOND:** if you can rule out critical illness most children do NOT need antibiotics
- **THIRD:** Need new diagnostic tools to improve triage and management of critically ill child

Severe Malaria: Triage

*PLoS ONE 2011;6:e17053*

- WHO criteria for the diagnosis and management of severe malaria (SM)
- Surveyed 105 health centers in Uganda for treatment of SM
  - Referral practices for SM were appropriate in less than 10% of cases
- Prompt care for SM → 29%
- SM diagnosed correctly → 27%
  → “...patient triage and evaluation were extremely inadequate”
Artesunate versus quinine in the treatment of severe falciparum malaria in African children (AQUAMAT): an open-label, randomised trial

Cerebral malaria fatality rates Artesunate vs quinine:
• Adult → 30% vs 39%
• Pediatric → 18% vs 21%

Blantyre Malaria Project Epilepsy Study (BMPES) of neurological outcomes in retinopathy-positive paediatric cerebral malaria survivors: a prospective cohort study

• Prospective cohort of CM vs UM controls x 18 m.
Summary:
→ 1/3 develop new motor, sensory or language deficiencies
→ 10% epilepsy
→ 10% behavioral disorders including ADHD
→ Neuroprotective interventions are needed
Diagnostics are not enough

**Critical Knowledge Gaps**

- **Who will get critically ill?**
- **Who needs referral? Admission?**
- **Who will benefit from ICU & high value therapeutics (HVTs)?**
- **How do we effectively allocate scarce resources to maximize impact?**

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**Solution:**

*Multiple Biothreats ➔ common pathway*

- Endothelial activation
- Vascular permeability ➔ Endothelial markers ➔ Identify those at risk of life-threatening infections

**Multi-organ failure: death***

*Despite antimicrobial treatment*

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**Host-based intervention**

* preserve endothelium integrity
* “agnostic” (pathogen-independent) countermeasure
Host response → severe and fatal disease
Host-based Rxs → augment/modulate innate/adaptive responses to improve outcome
Unique benefit of reducing antimicrobial resistance
Potential broad efficacy since pathway-based vs pathogen based
Can re-purpose FDA Rxs to accelerate RCTs

Impact (3 D’s)

**Save lives:** rapid triage for severe infections → interventions to stabilize endothelium will save lives

**Save brains:** protect BBB and neuronal injury → neuroprotective

**Save money:** prevent unnecessary referral, admission, drug use, toxicity, resistance, and misallocation of scarce health resources. Prevent brain injury.
**Angiopoietins**

**Angiopoietin-1**
- Good guy
- Binds and activates Tie2
- Mediates endothelial quiescence and vascular integrity

**Angiopoietin-2**
- Bad guy
- Blocks Ang-1
- Leads to endothelial activation and leak

**Endothelial biomarkers:**
- *Can we use endothelial biomarkers (e.g. Ang2) to predict at presentation who will become critically ill?*
- *Can we use biomarkers of this pathway to triage, risk stratify children and prognosticate who will die without immediate treatment?*
- *YES → endothelial/inflam markers (e.g. Ang2) predict outcome with high accuracy in multiple studies*
Angiopoietin-2 is an independent predictor of disease severity and survival

Does Ang-Tie2 at presentation of children with malaria predict:
- disease severity
- retinopathy
- subsequent in hospital mortality?

Biomarkers predict outcome in CM

Collaborators: T Taylor, K Seydel, M Molyneux, Malawi
Case-control: Malawian children presenting with CM (n=156) who subsequently died (n=60) vs survived infection

Retinopathy          Mortality (OR)

• Ang 1  5.9 (2.7-12.8)  2.4 (1.2-5.1)
• Ang 2  10.6 (4.6-24.6)  7.9 (2.6-23.6)
• sTie-2  11.7 (3.9-35)  3.2 (1.6-6.3)

Conroy et al Crit Care Med 2012
Angiopoietin-2 is associated with decreased endothelial nitric oxide and poor clinical outcome in severe falciparum malaria

Tsin W. Yeo\textsuperscript{a,b}, Daniel A. Lampah\textsuperscript{a,d}, Retno Gita\textsuperscript{a}, Emilliana Tjiitra\textsuperscript{a}, Emny Konangale\textsuperscript{a,c}, Kim Pisra\textsuperscript{a,b}, Ric N. Price\textsuperscript{a,b,c}, Stephen B. Duffull\textsuperscript{a}, David S. Celermajer\textsuperscript{a}, and Nicholas M. Anstey\textsuperscript{a,c,d}

Ang2
Independent quantitative predictor of disease severity and mortality

<table>
<thead>
<tr>
<th>Country</th>
<th>Thailand</th>
<th>Uganda</th>
<th>Malawi</th>
<th>Thailand</th>
<th>Papua</th>
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<tbody>
<tr>
<td>Study Population</td>
<td>Adults (≥13 yr)</td>
<td>Children (3-13yr)</td>
<td>Children (6mo-14yr)</td>
<td>Children (6mo-12yr)</td>
<td>Adults (≥13 yr)</td>
<td>Adults (7/age range)</td>
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<td>Sample size</td>
<td>10 HC</td>
<td>28 HC</td>
<td>53 UM</td>
<td>155 SM</td>
<td>70 UM</td>
<td>30 fatal</td>
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<td>25 UM</td>
<td>72 UM</td>
<td>44 CM</td>
<td>59 SMA</td>
<td>89 CM</td>
<td>CM/SM</td>
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<td>25 CM</td>
<td>69 CM</td>
<td>24 CNS disease</td>
<td>59 SM</td>
<td>36 SM</td>
<td>32 non-fatal</td>
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</table>

Principal findings
- Ang-1 is dec. and Ang-2 is inc. in patients with CM
- Low Ang-1 levels predict death in children with malaria
- Ang-1 and Ang-2 discriminate between UM and strictly defined CM with retinopathy
- Elevated Ang-2 and sTie-2 at presentation predict death in SM
- Combining biomarkers improves their prognostic value
- Elevated Ang-2 associated with SM and predicts death
- Elevated Ang-2 associated with pure CM, ARF, jaundice and predicts death

Ang-Tie2 as Biomarkers for Severe and Cerebral Malaria (n≥15)
Biomarker Kinetics

- Biomarkers: Predict response to therapy and outcome


Biomarkers predict 6 m mortality

- Biomarkers: Endothelial dysfunction and coagulopathy

Ang-Tie2 axis
Potential Applications to Patient Care

• Biomarker for Triage of Life threatening infections ➔ e.g. severe malaria, sepsis, ARDS/ALI, TSS, HUS etc.
• Evidence-based decisions ➔ referral, “Medivac”, admission, ICU monitoring
• Risk-stratify patients ➔ most likely to benefit from high value therapeutics
• Monitor response to therapy ➔ enable EBM-decisions on admission, ICU, antimicrobials
• Prognostic ➔ predict outcome and inform resource allocation

• N=2100 consecutive febrile children
• All cause febrile morbidity and mortality
Developing a prediction model for all cause febrile mortality

*Best clinical variables and biomarkers*

Adjustment for confounders, mediators, interaction variables...

*Minimal Sufficient Adjustment Set:* Abx, Age, HIV, IV, fluids, Malaria, Malaria_Tx, Pneumonia, Severity of Illness, Sex

http://dagitty.net/dags.html?id=svkxmD

Ang-Tie2 interventions:

- **Can we target this pathway to improve survival and prevent neurocognitive injury?**
- **Are there FDA-approved for unrelated indications that can be re-purposed to accelerate clinical translation and impact?**
  - **YES**
  - **Pro-Ang1 – Phase I**
  - **iNO – Phase II**
  - **PPARs – Phase II**
Ang-Tie2 and endothelial dysfunction

Intervention Strategies


PPARγ Agonists Improve Survival and Neurocognitive Outcomes in Experimental Cerebral Malaria and Induce Neuroprotective Pathways in Human Malaria

Lena Serghides1,2,3, Chloe R. McDonald1,2,3, Ziyue Lu1,2,3, Miriam Friedel1, Cheryl Cui1, Keith T. Ho4, Howard T. J. Mount5,7, John G. Sled8,9, Kevin C. Kain1,2,3

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Abstract

Cerebral malaria (CM) is associated with a high mortality rate, and long-term neurocognitive impairment in approximately one third of survivors. Adjunctive therapies that modify the pathophysiological processes involved in CM may improve outcomes over anti-malarial therapy alone. PPARγ agonists have been reported to have immunomodulatory effects in a variety of disease models. Here we report that adjunctive therapy with PPARγ agonists improved survival and long-term neurocognitive outcomes in the Primaquine berghei ANKA experimental model of CM. Compared to anti-malarial therapy alone, PPARγ adjunctive therapy administered to mice at the onset of CM signs, was associated with reduced endothelial activation, and enhanced expression of the anti-oxidant enzymes SOD-1 and catalase and the neurotrophic factors brain derived neurotrophic factor (BDNF) and nerve growth factor (NGF) in the brains of infected mice. Two months following infection, mice that were treated with anti-malarials alone demonstrated cognitive dysfunction, while mice that received PPARγ adjunctive therapy were completely protected from neurocognitive impairment and from PMA-infection induced brain atrophy. In humans with P. falciparum malaria, PPARγ therapy was associated with reduced endothelial activation and with induction of neuroprotective pathways, such as BDNF. These findings provide insight into mechanisms conferring improved survival and preventing neurocognitive injury in CM, and support the evaluation of PPARγ agonists in human CM.
PPARs improve survival over artemesunate alone


PPAR adjunctive therapy enhances Ang1, endothelial stability and BBB integrity

Rosiglitazone protects against brain injury

PPARs induce neurotrophic and neuroprotective factors during experimental cerebral malaria
PPARS confer protection to CM-induced neurocognitive impairment

Treatment protocol for behavioural studies (at CM onset with drug cure)

<table>
<thead>
<tr>
<th>Days</th>
<th>PbA</th>
<th>Artesunate (100mg/kg)</th>
<th>Mefloquine (15mg/kg)</th>
<th>Rosiglitazone (2.5mg/kg)</th>
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Behavioural testing

MRI imaging

Novel Object Recognition test – learning, memory, attention

Rotarod test – motor function


PPARS protect against from malaria-induced neurocognitive impairment

PPARS protect against from malaria-induced brain atrophy


Use of Peroxisome Proliferator-Activated Receptor γ Agonists as Adjunctive Treatment for Plasmodium falciparum Malaria: A Randomized, Double-Blind, Placebo-Controlled Trial

Andrea K. Boggild,†,‡ Srivicha Krudsood,†,‡ Samir N. Patel,†,‡ Lena Serghides,†,‡ Noppadon Tangpechdee,§ Kevin Katz,§ Polrot Wilaivivat,¶ W. Conrad Liles,∥∥∥ Sorachai Laosareewong,∥∥∥ and Kevin C. Kain†,‡,∥∥∥

†Tropical Disease Unit, Toronto General Hospital of the University Health Network, ‡Department of Laboratory Medicine and Pathobiology and ‡Division of Infectious Diseases, Department of Medicine, University of Toronto, and ¶McLaughlin-Rotman Centre for Global Health, Toronto General Hospital, McLaughlin Centre for Molecular Medicine, University of Toronto, and §North York General Hospital, Toronto, Ontario, Canada. •Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

(See the editorial commentary by Shanks, on pages 850-1.)

PPARγ Agonists and Malaria • CID 2009:49 (15 September) • 841
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>
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<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>3 - 72</td>
<td>3 - 75</td>
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<tr>
<td>Parasite clearance time 90% (h)</td>
<td>30.9 (18.2)</td>
<td>40.4 (21.9)</td>
<td>0.0035</td>
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<tr>
<td>Mean (SD)</td>
<td>5 - 78</td>
<td>5 - 84</td>
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<tr>
<td>Range</td>
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Boggild et al. 2009

Rosiglitazone reduces parasite burden in patients with malaria → AMR

Boggild et al. 2009
Rosiglitazone reduces levels of inflammatory biomarkers in patients with malaria

IL-6

MCP-1

TNF

Boggild et al. 2009

PPARs stabilize endothelium and induce neuroprotective factors in humans

BDNF is associated with neurological severity and neurocognitive outcomes in Ugandan children with severe malaria (n=180)

**Summary**

- The current inability to rapidly identify SM is a barrier to improved outcomes and reduced health costs
- Adding biomarkers to clinical scores improves predictive accuracy or can replace scores
- As few as 1 biomarker with a pathophysiologic link to severe infection can reliably risk-stratify febrile children for all cause febrile mortality
- These markers are “druggable” targets
- Cost benefit analysis indicates this strategy can save lives and save money

*McDonald C et al. 2016 (in press)*