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Strategies to perform and manage severe infection studies in mice: lessons from cerebral malaria

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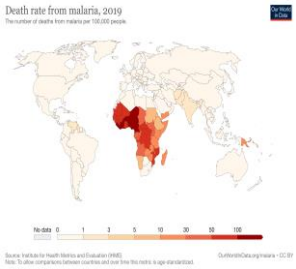
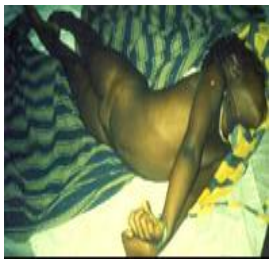
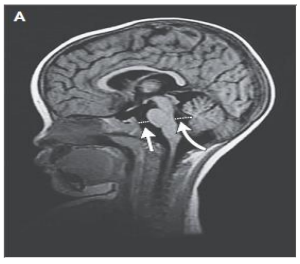
Cerebral malaria: the most severe complication of malaria

- Syndrome affects ~ 2 million people annually, causing death of ~ 400,000 young children each year
- A very rapid onset syndrome that can cause death in 24h
  - Lack of prognostic symptoms or biomarkers prevents prophylactic intervention
- No specific treatments for the syndrome, only anti-malarial drugs
  - These are ineffective in 20% of cases
- 15-20% of survivors are left with long-term neurological deficits
  - Blindness, epilepsy, learning difficulties, behavioural problems
- Inaccessibility of the brain means animal models are required to study the syndrome

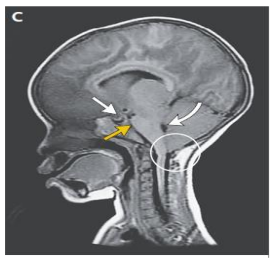




normal



Cerebral malaria

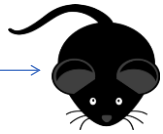


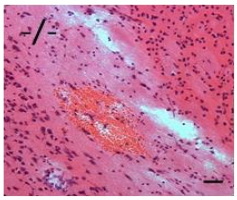
The *Plasmodium berghei* ANKA (*Pb ANKA*) murine model of Experimental Cerebral Malaria (ECM)

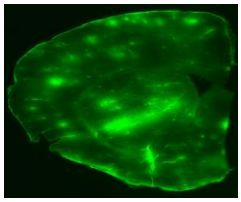
- *P. berghei* ANKA infection of C57BL/6 mice
  - First utilised in 1982
- Causes comparable pathological events in brain as observed in humans with CM
  - Includes haemorrhage, oedema, neuronal damage, brain swelling
- Anti-malarial drug treatment has similar sub-optimal efficacy as in human cerebral malaria
  - Unsuccessful in ~20% of cases, with surviving mice frequently displaying behavioural problems
- ECM is a valuable tractable model to develop new therapies for the CM syndrome
  - If used appropriately to mimic the clinical situation

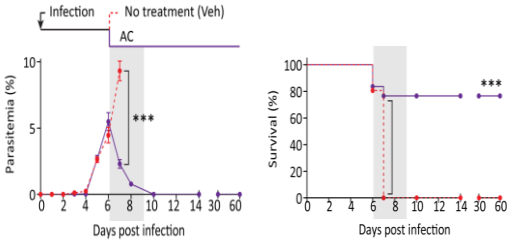
Strangward *et al* PLOS Pathogens 2017; Strangward *et al* PNAS 2018

Plasmodium berghei ANKA



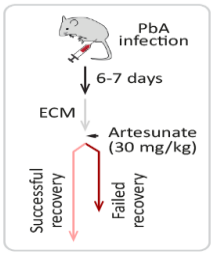


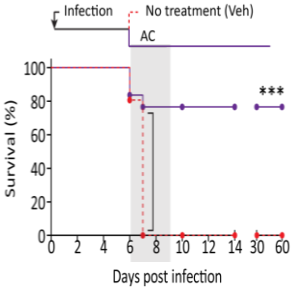


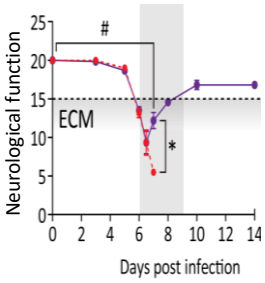


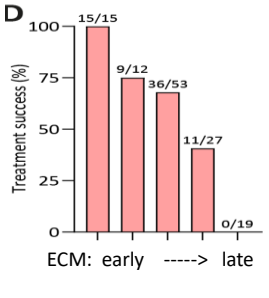
The experimental challenges of working with the ECM model

- It is a fast onset, rapidly progressing, syndrome that is fatal without treatment
  - Fatal within ~24h after initial signs of disease
- To identify pathways controlling recovery from ECM, we must let the syndrome develop
  - Need a robust system to rapidly identify mice at correct stage of disease for experimentation, but prevent unnecessary suffering
- We must be able to accurately assess the efficacy of treatments
  - Need to quantitatively assess if a treatment is protective
- Variability in timing of syndrome onset frequently occurs within groups of mice in experiments
  - Need to quickly and non-invasively assess each mouse (sometimes >20 mice in an experiment), so each mouse is treated at correct stage









Defining the adverse effects and humane end points of experimental cerebral malaria

We have devised the following clinical scale of disease to monitor the stepwise progression of ECM development:

Non-specific
1. no signs
2. Slight abnormal posture (slight hunching) +/- hyperactivity (rapid and continual movement around cage)
3. slight hunching + ataxia (controlled)

prodromal
4. slight hunching + ataxia (less balanced but controlled) + slower spontaneous movement around cage
5. Hunching (more pronounced) + ataxia (wobbly with side-to-side movement) + lethargy (responsive to stimulation and will move if touched or lifted and replaced in cage but not moving normally around cage) + / - hyperventilation (potentially shallow and accelerated breathing)

ECM
6. Hunching (severe but upright tucked into ball) + ataxia (slow with very unsteady and shaking walk) + not moving and reduced response to stimulation (for example ear tap or touch on side) + respiratory distress (deep and laboured breathing). Fully reversable upon treatment if less than 12h in duration
7. prostration (lying and unable to reorientate if placed on side) + unable to walk
8. Hind leg paralysis and /or convulsions and / or coma
9. death

We require mice to progress to stage 6 for experimentation but aim to avoid progression to stage 7-8

The stringent monitoring of mice with ECM

To ensure we identify mice at the correct stage of disease, we have developed and refined a specific monitoring system, determined by our knowledge of the progression speed of the ECM syndrome aligned with the grades in our clinical scale:

	Stage	Duration	Monitoring Frequency	End Point
N-specific	1.		1X Daily	
	2.	6-15h (older mice progress slower)	Every 6h	
	3.			
prodromal	4.	6-12h	Every 4h (including through night)	
	5.			
ECM	6.	6h	Every 4h	Immediate (<12h if treated)
	7.	3h	N/A	Immediate
	8.	1-2h	N/A	Immediate

With this monitoring system, <10% of mice on the PPL experience unnecessary suffering (less than 4h in duration), and clear end-points are established for researchers and animal caretakers

Further refinement: The Rapid Murine Coma and Behavioural Scale (RMCBS)

- Our clinical grading system robustly identifies mice with ECM but is qualitative and doesn't fully capture subtle neurological alterations, or some behavioural changes
- In humans, CM is quantitatively graded using the Blantyre coma scale
  - Assesses eye movement, motor and verbal responses
- The RMCBS was developed to quantitively grade ECM development, akin to situation in humans
  - A powerful method to assess treatment efficacy
- Based upon SHIRPA protocol but much faster
  - ~3 min per mouse for 10 parameter RMCBS vs 20-30 min for 40 parameter SHIRPA test
  - Linear regression modelling identified the key predictive parameters to include within RMCBS

RMCBS measurements

Label	Score
Coordination	
Gait	(0–2)
Balance	(0–2)
Exploratory Behavior	
Motor Performance	(0–2)
Strength and Tone	
Body Position	(0–2)
Limb Strength	(0–2)
Reflexes and Self-Preservation	
Touch Escape	(0–2)
Pinna Reflex	(0–2)
Toe Pinch	(0–2)
Aggression	(0–2)
Hygiene-Related Behavior	
Grooming	(0–2)

0 = suffering 2 = normal Carroll *et al* PLOS One 2010

The Rapid Murine Coma and Behaviour Scale (RMCBS)

Coordination
Gait (0–2) (none – ataxic – normal)
Balance (0–2) (no body extension – extends front feet on wall – entire body lift)

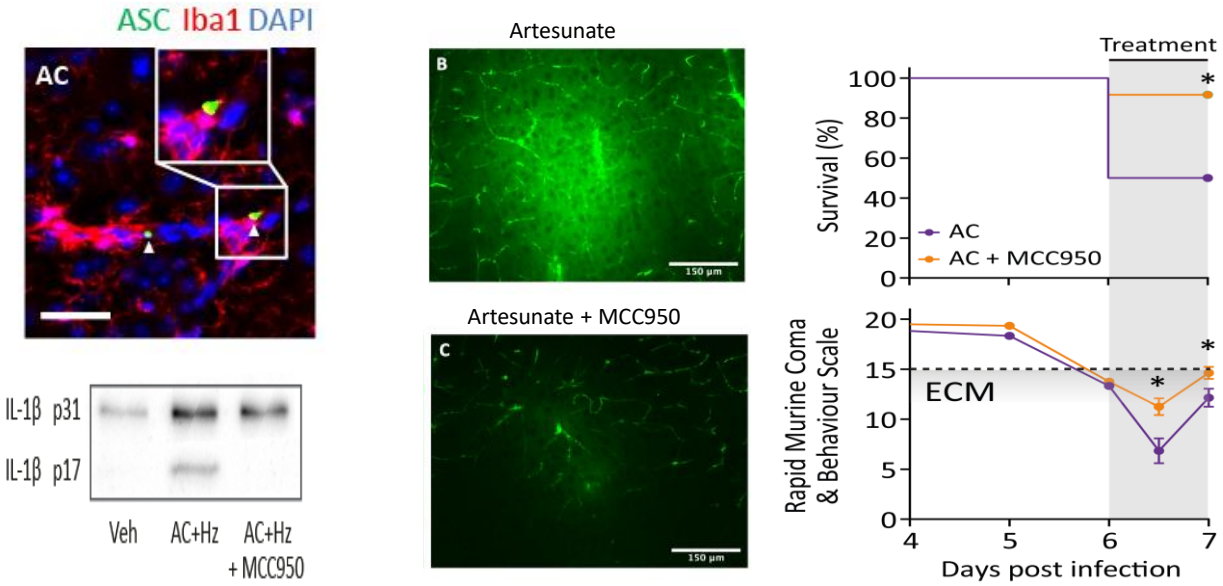
Exploratory Behaviour
Motor Performance (0–2) (none – 2–3 corners explored in 90 seconds – explores 4 corners in 15 seconds)
Strength and Tone Body Position (0–2) (on side – hunched – full extension)
Limb Strength (0–2) (hypotonic, no grasp – weak pull-back[front paw grasp only] – strong pullback[active pull away, jerk away])

Reflexes and Self-Preservation
Touch Escape (0–2) (none – unilateral – instant and bilateral; in 3 attempts)
Pinna Reflex (0–2) (none – unilateral – instant and bilateral; in 3 attempts)
Toe Pinch (0–2) (none – unilateral – instant and bilateral; in 3 attempts)
Aggression (0–2) (none – bite attempt with tail cut – bite attempt prior to tail cut, in 5 seconds)

Hygiene-Related Behaviour
Grooming (0–2) (ruffled, with swaths of hair out of place – dusty/piloerection – normal/clean with sheen)

Carroll *et al* PLOS One 2010: https://doi.org/10.1371/journal.pone.0013124 (includes video showing performance of RMCBS)

Identifying potential new treatments for CM: the NLRP3 inflammasome



Summary

- Validated and clinically-relevant animal models are essential for development of new treatments for cerebral malaria
- The nature and progression speed of the ECM syndrome means robust animal monitoring is critical to avoid unnecessary suffering
- Established animal assessment protocols (such as SHIRPA), can be adapted for rapid and quantitative monitoring and grading of ECM (and other neurological diseases)
- Utilising these refined grading systems, we have made substantial progress in understanding the pathways that control recovery from ECM

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