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The materials in this document are intended as general medical information and are not intended to constitute a recommendation as to a course of medical treatment for any individual patient. They are provided for the limited purpose of assisting clinicians as they evaluate available treatment options. These materials represent the insights and opinions of physicians involved in treatment of patients with rabies and are not the result of activities pursuant to an approved research protocol, and they should be evaluated on that basis. The information provided in this document is based on a very limited experience and therefore may not be applicable in any other situation. Each rabies patient is unique, and factors such as general good health, excellent and adaptive medical intensive care, and careful avoidance of mistakes and complications of intensive care may prove to be essential to positive outcomes.

The information, including the identification of key issues, and the recommendations provided remain preliminary in nature. As noted, they do not constitute the current standard of care. This document will be modified as additional data is accumulated. The risks associated with the course of treatment described generally in these materials must be understood and carefully evaluated by physician and patient before treatment decisions are made. Any additional information that other clinicians or researchers may provide related to the treatment of rabies in other patients is greatly appreciated.

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STOP!

What to do first*:*

DO NOT:

- **DO NOT administer rabies vaccine or immunoglobulin**. Section D.2
  This has never worked in rabid patients.
  It violates a key assumption of the Milwaukee protocol.
  It may cause "early death" phenomena, based on case reports and animal models.

- **DO NOT begin the Milwaukee protocol until the diagnosis is confirmed.** Section B
  The protocol is very aggressive and may result in complications without prospect of benefit.
  We DO recommend sedation to prevent life-threatening cardiac dysrhythmias – ***see next page***

- Rabies is NOT likely in patients:
  - Without a fever
  - With an illness lasting more than 14 days (other than Guillain-Barre-like syndrome)
  - With an incubation period following an animal bite or transplantation of < 10 days or > 1 year
  - Who completed a full course of rabies post-exposure prophylaxis including immune globulin

- The Milwaukee Protocol assumes normal immunity and no irreversible medical complication.
  - It may not be as effective for those < 5 years or > 55 years of age.
  - It may not be as effective for those receiving immune suppression.

Please note

Rabies mimics brainstem death clinically, reversibly.(1)
Rabies causes sensory denervation, like Miller-Fisher syndrome and Birkenstaff encephalitis.
Peripheral nerve dysfunction results in paresis.(2)
Rabies includes papillary abnormalities (fixed dilated, anisocoria) as part of dysautonomia.(2)

Rabies causes loss of EEG amplitude, reversibly

- **Generalized vasospasm of the cerebral arteries occurs around day 6-8 of hospitalization, and may cause profound drop in EEG amplitude.(3)**

EEG has returned with pharmacological improvements in blood velocity.

Corollary: Standard criteria for brain death do not apply. Diagnosis of brain death requires anatomic (biopsy) evidence for irreversible brain damage or a brain flow scan showing zero flow intracranially.

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A laboratory-confirmed diagnosis of rabies is essential. Once rabies is considered:

DO:

1. **Isolate the patient** to minimize the number of medical staff who might require later prophylaxis.

2. Minimize early demise in 20% of patients from catecholamine storm or bradyarrythmia/asystole.
   - **Minimize stimulation** – similar to management of tetanus.
   - **Use heavy sedation (preferably midazolam ± fentanyl)** x 24 h.
     Avoid barbiturates – these inhibit the immune response.
     Propofol has a relative contraindication.
     You can confirm the diagnosis, or have strong negative prediction by 24h of receipt of samples by CDC or other rabies reference laboratory. If not rabies: sedation can then be withdrawn.

   - Consider placement of **EXTERNAL pacing wires** in anticipation of asystole.
     Asystole often responds to increased sedation.

3. If your patient is extremely **agitated, hypertensive, with tachyarrhythmia**, consider diagnosis of CNS-mediated catecholamine storm. This may cause cardiomyopathy if untreated. **Bradyarrhythmias and asystole** are also common.

4. If your patient has **hypotension**, then there is likely hypovolemia.
   - Consider **volume replacement using normal saline before vasopressors**.
   - Consider **measurement of CVP**.
   - *Vasopressors may be needed but are relatively contraindicated because vasoconstriction is unopposed in rabies when BH4 deficiency causes loss of NO-mediated dilatory tone of the cerebral circulation. Cerebral vasospasm will not necessarily be evident clinically (similar to vasospasm after subarachnoid hemorrhage)*

5. **Confirm the diagnosis**. You need serum, cerebrospinal fluid (CSF), saliva and biopsy of hairy skin. Call CDC rabies branch (+1 404 639 1050) or your national rabies reference laboratory to request assistance

   www.cdc.gov/ncidod/dvrd/rabies/professional/Prof.forms/antem.htm

   Diagnosis can be confirmed within 6 hours of receipt of overnight package.
   Be sure to properly label (name, tissue) and date all specimens!

6. Establish exact dates (these are needed for your consultants to guide patient management):
   Date of animal bite or exposure
   Date of prodromal symptoms (onset)
   Date of first objective signs (usually this is date of hospitalization)

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7. Plan your logistics [See downloadable Checklist & Schema at www.mcw.edu/rabies]

- Rabies care can be accomplished in **hospitals capable of treating tetanus, Guillain-Barre syndrome and/or head trauma.** Consider transfer to leading referral hospitals.
- You need regular access to rabies serology from a **rabies reference laboratory**.
- You need to have access to excellent **rehabilitation specialists**.

- **Obtain drugs – See checklist**.

- You will **need continuous EEG or Bispectral Index (BIS) monitoring** to care for rabies. Plan for 1-3 weeks of monitoring. BIS monitors are used in anesthesiology.

- Get **baseline transcranial Doppler ultrasound (TCD)** of middle cerebral arteries
  Rabies leads to cerebral artery vasospasm causing decline in EEG and seizures. This is not evident clinically until very late (similar to DID after subarachnoid hemorrhage)
  Plan for 2 weeks of daily monitoring
  CT angiography is indicated for confirmation of severe spasm by TCD.

- **Contact Molecular Neurogenetics to request 3 collection kits** for biopterin & neurotransmitter analyses of cerebrospinal fluid, and notify them of imminent need for rapid reporting for an active case of rabies. +1 678.225.0222 www.medicalneurogenetics.com
  Contact us for other reference laboratories around the world: rabies@chw.org

- **Call Neurosurgery**. It has been very difficult to obtain prompt neurosurgical intervention. It is strongly recommended that neurosurgery be consulted soon after diagnosis in order to familiarize the surgeons with recent advances in treatment of rabies, such that considerations of futility and fear of rabies can be addressed ahead of any emergent need for their services.

- **Call Neuroradiology** for anticipated requirements for CT angiography around day 6-10 and day 13-17 in association with generalized cerebral vasospasm.

Drugs with relative contraindications in rabies (mostly based on anecdote)		
Barbiturates	Suppress immunity, that we require for cure	Section B
Propofol	Has led to acutely flat EEG in 2 patients.	Section B
Topiramate	Has led to acutely flat EEG in 2 patients.	Section B
Vasopressors	May lead to unopposed vasoconstriction with BH4 deficiency	Section E
Diuretics	Confound management of SIADH, DI, salt wasting and subacute cerebral edema	Appendix II

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THEORY

Original assumptions(4)

1. Rabies is without viral or immune-mediated cytopathic effect, therefore reversible.
2. Clearance of rabies requires *normal immunity.*

New evidence (protocol version 2)

A. Acquired tetrahydrobiopterin (BH4) deficiency in CSF is found regularly in human rabies.(5)

B. Generalized cerebral artery spasm is found regularly in human rabies.(3)

New evidence (protocol version 3)

C. Calcium-channel blockers, used prophylactically, may avoid vasospasm and metabolic complications of rabies, such as progressive CSF lactic acidosis.

D. Ribavirin results in delayed and depressed immunological responses to rabies and so is contraindicated.

E. Strong immune responses to rabies are associated with subacute and prolonged cerebral edema. The cerebral edema appears to be neither cytotoxic nor vasogenic – it may be "cytomegalic". It responds to induced hypernatremia. *This complication may be more common in bat-associated rabies.*

F. Elevated levels of quinolinic acid are found in CSF from very early times in human rabies. Quinolinic acid is excitotoxic and can be treated by NMDA blockade (ketamine).

Added assumptions:

3. The protocol is followed closely -- all protocol drugs are used.
4. BH4 deficiency is in the causal pathway for rabies.
5. Generalized cerebral vasospasm is in the causal pathway for rabies.

The protocol drugs have multiple pharmacological actions, so changes may have unintended consequences. Outside of clinical trials, well intentioned “improvements” are *neither scientific nor in the patient’s best interest. The pathophysiology of rabies is unique and counter-intuitive relative to other conventions of critical care.*

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A. Supportive care

Rationale

For almost 40 years, clinicians have speculated that survival from rabies was possible given meticulous, intensive medical support. This has only been documented for confirmed rabies on six occasions.(6)

- Supportive care in a dedicated rabies intensive care unit (ICU) under Gode permitted 2 survivals (virologically unproven) out of 37 patients treated with diphenylhydantoin and ascorbate.(7)
- Supportive care in the United States from 1960 to 1979 resulted in 2 survivors out of 38 patients.(8)

Standard antivirals, classically including immune modulators (alpha-interferon), do NOT work.(9)

- There were no survivors of 17 patients treated with antivirals by Gode in the same unit that reported survivals with supportive therapy (above). Antiviral therapy varied and was not specified.
- Aggressive critical care with antiviral therapy by Warrell resulted in no survivors in 5 attempts.(10)
- Use of combination antiviral therapy has shown mixed outcomes.(4;11) It is clear from early attempts at the Milwaukee Protocol (v.1) that there is no antiviral effect of ribavirin, ketamine, and amantadine.

Late demise after rabies is associated with clearance of the rabies virus at necropsy, indicating that a normal immune response is sufficient to clear rabies virus if survival can be prolonged sufficiently.(12;13) There is a clearance of viral genome in saliva with advent of rabies neutralizing antibody in serum.

A.1 Anticipation of problems and gauging of medical progress (Appendix II)

There are several excellent case reports that provide detailed information on when medical complications occur during care of rabies.(14) A partial listing of anticipated complications is also useful in gauging progress toward survival, as common complications are avoided or minimized. See Figure.

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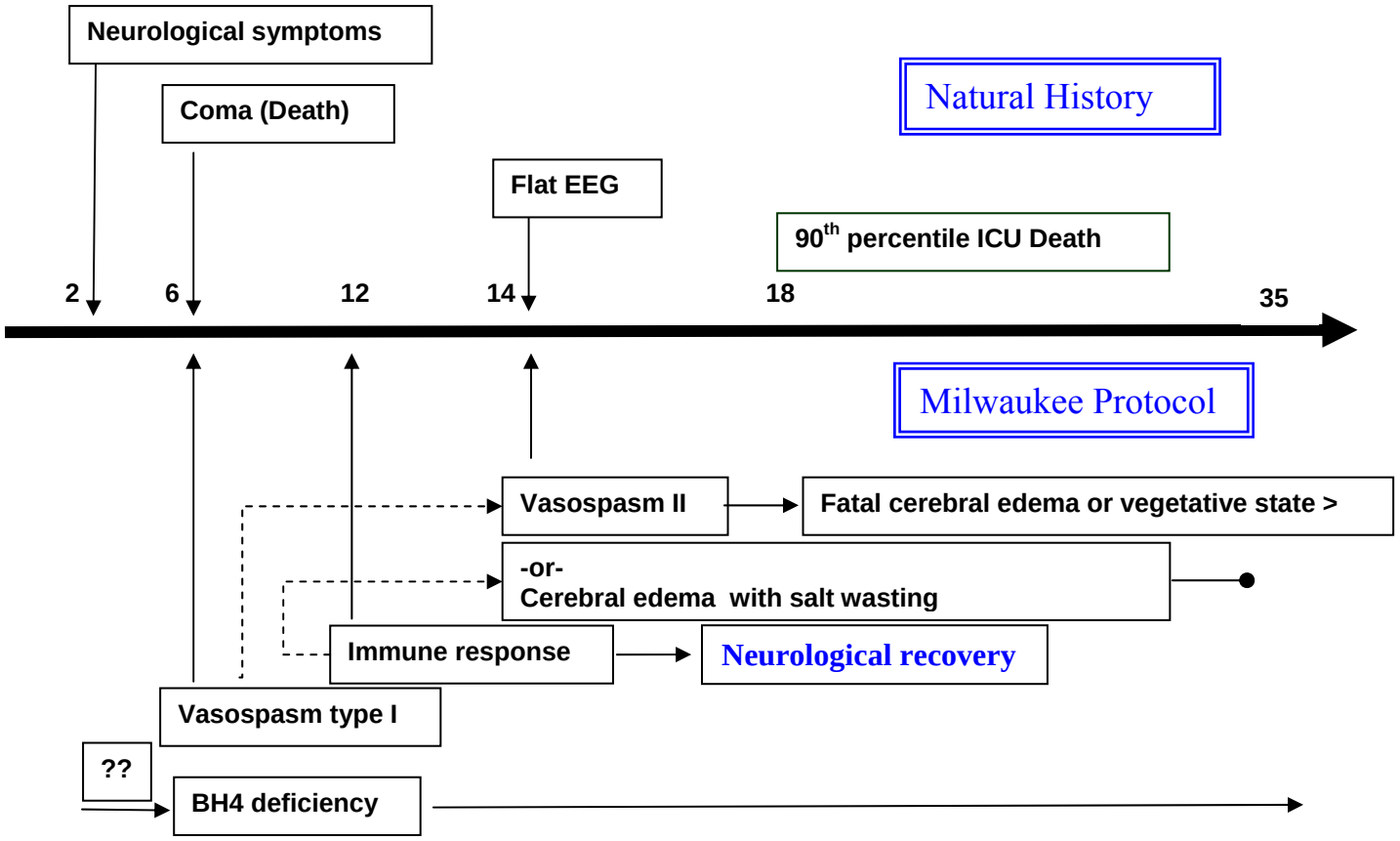

A.2 Supportive care: Palliation

Rationale

Current standard of care is palliation, focused on minimization of environmental stimuli that provoke hydrophobic or aerophobic spasms, and relief of anxiety and pain.(9) The single formal study of palliation investigated haloperidol, diazepam and diphenhydramine.(15)

Milwaukee protocol also accomplishes these palliative goals.

1. Ketamine (a dissociative anesthetic) at 1-2 mg/kg/h
2. A benzodiazepine (diazepam, alprazolam or midazolam) to balance the metabolic and hallucinatory effects of ketamine.

Key point 1: Aggressive sedation and analgesia are effective at preventing or reversing severe dysautonomia that may kill the patient.

Key point 2: We recommend that deep sedation or therapeutic coma be implemented for at least 7-10 days without interval tapering of the drugs to permit interim neurological examinations. Neurological examinations are not informative based on the known biology of rabies, while dysautonomia may be fatal. (See A.4. Denervation and misinterpretation of brainstem death.)

By 7-10 days, there is usually evidence of denervation of the autonomic nervous system, so that lethal brain-mediated dysrhythmias are less likely and sedation can be tapered.

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Key point 3. There is nothing magical about burst suppression by EEG. The goal is avoidance of dysautonomia. It is NOT advisable to reach full burst suppression because it is hard to titrate sedation once the EEG is fully suppressed. The EEG also serves as an early indicator of severe arterial spasm (see E.2. Biopterin deficiency in the CNS will reduce cerebral perfusion.).(16)

A.3 Supportive care: Fluids, electrolytes, neuroendocrine

We recommend maintenance of euvolemia.

A.3.1 Rationale v. complication of SIADH

Patients often are dehydrated because of fever and hydrophobia. The hypothalamus is regularly affected during rabies and disorders of water metabolism are common.

Classically, there is an initial period of excessive secretion of antidiuretic hormone (ADH), followed within 1-3 days by diabetes insipidus (DI). The syndrome of inappropriately high antidiuretic hormone (SIADH) is relatively subtle but must be recognised to avoid cerebral edema. (see A.10. Cerebral edema and brain perfusion pressure).

ORIGINAL PROTOCOL:

1. The patient should be given normotonic saline or equivalent crystalloid to correct dehydration.
2. Serum sodium should be measured twice daily.
3. Urine output should be assessed every 4-6 hours. Monitor urine specific gravity.
4. Restriction of free water is effective at treating SIADH.
5. Central venous pressure was targeted to the normal range.

A.3.2 Rationale v. complication of DI

We have only encountered severe DI in rabies in the setting of severe complications, such as catastrophic stroke. Diabetes insipidus can be severe, prolonged, and periodic.

Because ADH is a potent suppressor of the fever response, DI may be associated with hyperthermia.

Bolus administration of vasopressin to treat DI may exacerbate hypertension during dysautonomia and also cause unopposed vasospasm of cranial arteries following rabies-associated BH4 and NO deficiency. Continuous dosing on a sliding scale is recommended, along with careful monitoring of intracranial blood flow. Consider supplementation with 2 mg/kg/day of oral BH4 during vasopressin replacement. (See E. 2. Biopterin deficiency in the CNS will reduce cerebral perfusion.)

MODIFIED PROTOCOL (version 2.1)

During DI, we recommend mL/mL replacement of urinary output above 2 mL/kg/hour output with:

1 milliUnit arginine vasopressin/500 mL of D2.5, 0.2NS (made as 250 mL D5W + 250 mL 0.45 NS. Final AVP = 0.2 milliU/mL replacement fluid), replaced every 2-4 hours.(17) Max dose 10 milliU/kg/h
[Calculation: replacement of excess at 1 mL/kg/h = 0.2 milliU/kg/h = 0.0033 milliU/kg/min]
[Reference dosages: GI bleed 2-5 milliU/kg/min; shock doses: 0.02 – 2.0 milliU/kg/min]

Caution: steady state drips as low as 0.05 milliU/kg/h were excessive in treating DI in one case.

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A.3.3 Rationale for monitoring hypothalamic-pituitary axis

Many species of animals show fatal deficiencies in growth hormone during early rabies.(18) Cortisol and thyroid hormones are not affected absent complications. We diagnosed true thyroid deficiency in one patient in week 3, after a cardiac arrest in week 1. Negative cortisol stimulation test was documented in one patient in week 1, after a cardiac arrest.(14)

Unfortunately, interpretation of growth hormone-somatostatin-insulin-like growth factor axis and thyroid hormone levels during critical illness is problematic, and supraphysiologic replacement of growth hormone may be associated with increased fatality.(19)

MODIFIED PROTOCOL, (version 2.1)

1. Consider weekly monitoring for deficiencies in thyroid, cortisol, and growth hormone. Replacement is reserved for only severe deficiencies.
2. Consider weekly monitoring for serum prolactin that may provide a useful index of hypothalamic integrity and brain dopamine metabolism (see A.5 Evidence of metabolic deficiencies).(20)
3. Evaluate thyroid, cortisol, and growth hormone axes when DI or refractory hypotension is encountered.

A.4. Denervation and misinterpretation of brainstem death

Rationale

Rabies is clinically similar to Bickerstaff encephalitis or Miller-Fisher variant of acute inflammatory polyneuropathy (AIDP, Guillain-Barre-Landry syndrome). The pathogenesis of rabies includes (anterograde) emigration of the rabies virus from the brain along motor, sensory and autonomic nerves. Universal dysfunction of peripheral nerves may explain signs of organ denervation and motor, bulbar and diaphragmatic paresis.(4) Loss of pupillary function (dilated pupils, anisocoria) are early signs of dysautonomia in rabies.(2) Loss of corneal and oculovestibular reflexes is common, but may not necessarily reflect brainstem death because of the predicted sensory neuropathy. (1;4) Recovery from clinical denervation in one survivor was rapid, consistent with loss of function than true denervation.(4)

Note: We found absent VERs in one patient at a time when MRI anatomy and diffusion-weighted imaging, as well as blood flow by TCD, were normal.

MODIFIED PROTOCOL (version 2.1)

1. We recommend AGAINST suspending anesthesia to permit interim neurological evaluations during the first week of induced coma. Stimulation may result in cardiac asystole (see below A.6. Anticipation and prevention of cardiac arrhythmias and myopathy).
2. Once there is evidence for autonomic denervation -- evidenced by lack of heart rate and blood pressure variability over 24 hours -- it may be prudent to *very slowly* taper sedation.
3. The patient is best followed by assessing amplitude of the EEG tracing, or BIS number.
4. **It is unwise to push to complete burst suppression.** The intent is to suppress severe dysautonomia, and full burst suppression loses the capability to assess trends in EEG over time or in association with acute cerebral artery vasospasm.

   Consider visual evoked responses, nerve conduction studies and SSEPs to better elucidate their utility in assessing progression and recovery from rabies during induced coma. .
5. We anticipate full paresis by 10-14 days of rabies, with rapid recovery within 10-14 days of the brisk antibody response in the cerebrospinal fluid. (See Figure)
6. Brain biopsy or nuclear brain scans are probably the only way of proving futility of care in human rabies (see G. Duration of care/Clearance of virus).

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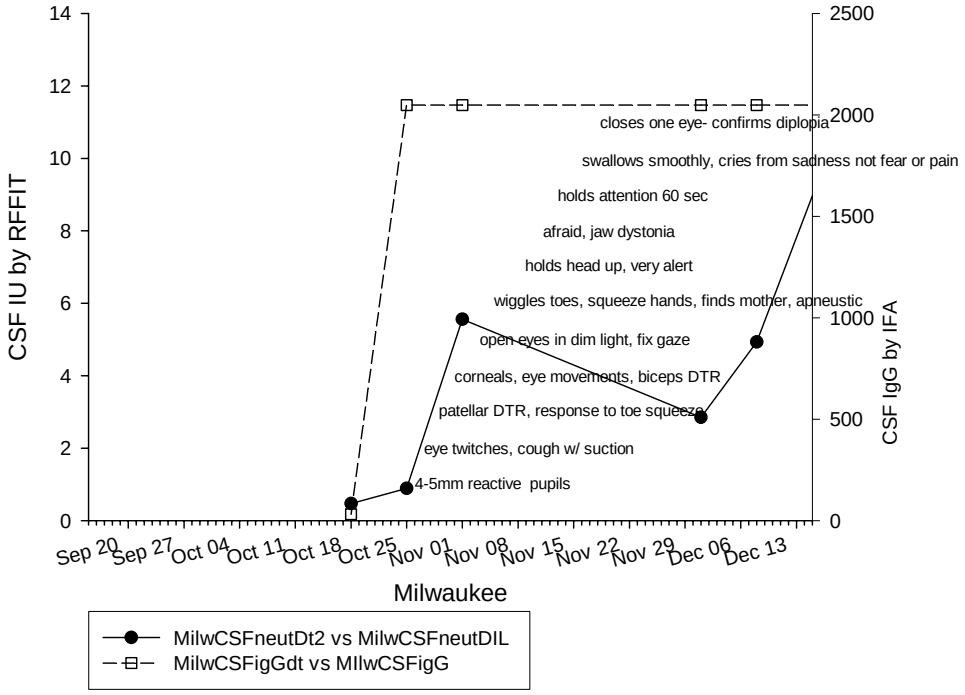

Figure showing Total rabies specific (dotted line) and neutralizing rabies antibody in cerebrospinal fluid relative to recovery of neurological function in the index survivor.

A.5 Evidence of metabolic deficiencies

Please refer to Section E for detailed information on biopterin deficiency associated with rabies.

Rationale

1. We encountered unexplained residual mild lactic acidosis, persistent tachycardia with depressed myocardial contractility, pancreatitis, a peripheral neuropathy (clinical diagnosis) and late basal ganglia hyperintensities on FLAIR sequences (MRI) during the first month of rehabilitation of the first survivor receiving the Milwaukee protocol. Depressed myocardial contractility is regularly described during the terminal phase of rabies, while the mechanism of the peripheral denervation associated with rabies is largely unknown and the electrophysiology is heterogeneous.(21)

We considered it unusual to have rapid recovery of most functions in our patient, with static deficits in other organ systems. The pattern suggested a mitochondrial disorder, and ribavirin, a nucleoside analog, is variably toxic to mitochondria (see below C.1. Ribavirin and vidarabine).(22). We therefore postulated an acquired mitochondrial disorder associated with either rabies or our treatment protocol. Proton spectroscopy of her brain showed low NAA concentrations diffusely, suggestive of a mitochondrial disorder. We empirically treated with a mitochondrial cocktail while tests were in process, and confirmed deficiencies of coenzyme Q10 (Q10) and tetrahydrobiopterin (BH4) in our survivor at that time.

- Two later patients with rabies did not have deficiencies of coenzyme Q10.
- Prophylactic administration of coenzyme Q10 at lower doses in patients with rabies has not prevented deficiency of BH4 in CSF. **Supplementation is no longer recommended.**

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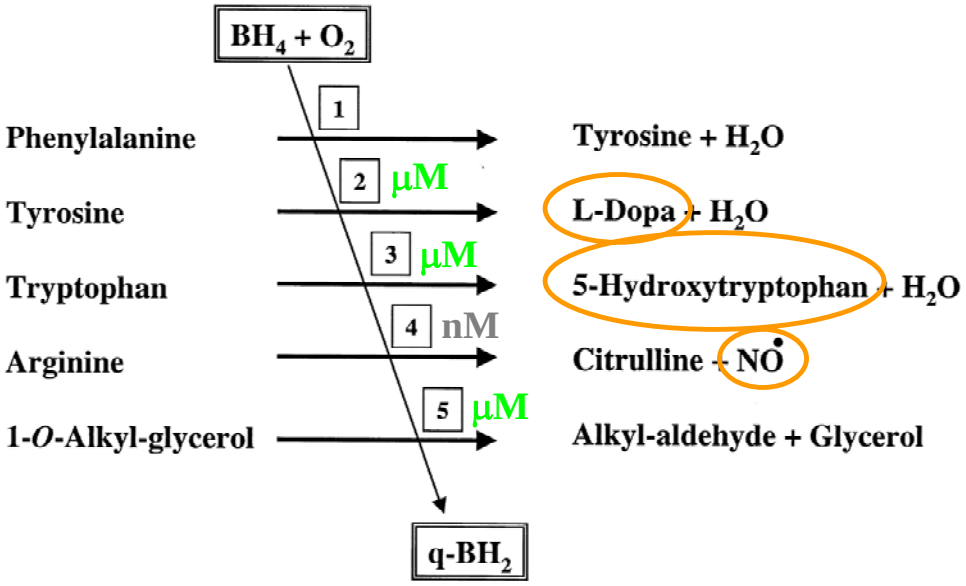
2. We have confirmed a deficiency in tetrahydrobiopterin (BH4) in the cerebrospinal fluid (CSF) of 5 of 5 patients with rabies treated with the Milwaukee protocol.(5) Another patient likely also had BH4 deficiency, but normal ranges for ventricular fluid are not available.

- Ribavirin, a guanosine analog, is predicted to lower biopterin levels by depleting stores of the biopterin precursor, GTP (see below C.1. Ribavirin and vidarabine)
  Note: One rabies patient, with BH4 deficiency in CSF, did NOT receive ribavirin. This effectively excludes such confounding.

There are 5 BH4-dependent enzymes:



1) BH4 is distributed for treatment of phenylketonuria. Rabies is neurotropic and should not affect the liver. There was no elevation of phenylalanine in the blood or CSF of our survivor at time of proven BH4 deficiency in CSF.
2) BH4 is essential for synthesis of catecholamine neurotransmitters, i.e., dopamine, norepinephrine, epinephrine. Km of BH4 for tyrosine hydroxylase is micromolar.
3) BH4 is essential for synthesis of serotonergic neurotransmitters, i.e., serotonin, melatonin. Km of BH4 for tryptophan hydroxylase is micromolar. In animal knockouts for BH4, serotonin remains detectable, suggesting that serotonin deficiency is incomplete.
4) BH4 is essential for the proper assembly and signal transduction of nitric oxide synthase (NOS) in the brain. BH4 is also oxidized enzymatically during NO synthesis to qBH2 and must be reduced back to active form. Altered ratios of BH4 to its stably oxidized metabolite 7,8-BH2 uncouples NOS, generates superoxide rather than NO, and leads to paradoxical vasoconstriction (so-called endothelial dysfunction).(23) Km of BH4 for NOS is nanomolar.
5) BH4 is an essential cofactor for alkyl-glycerol monooxygenase (AGMO). Alkyl-glycerol linkages are heavily enriched in brain plasmalogens and the metabolic precursor pool to platelet activating factor (PAF). PAF promotes neuronal necrosis and is very active as a mediator of anaphylaxis, vasogenic edema and vasodilatory shock, with vasoconstriction in select vascular beds (renal).(24) PAF is deacetylated to lyso-PAF that in turn is degraded by AGMO to a fatty aldehyde and glycerol. (The importance of this enzyme outside of liver and intestines is not clear. A second degradative pathway for lyso-PAF also exists.)(25) The Km of BH4 for alkyl glycerol monooxygenase is micromolar.

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3. Vitamin C is a cell-permeable antioxidant capable of preserving BH4 in its active form.(26) In a series of 37 patients with (laboratory-unconfirmed) rabies, treated prophylactically with diphenylhydantoin and vitamin C (ascorbic acid), there were 2 survivors.(7) It also has shown mild efficacy as a antiviral against rabies in animal models and as a laboratory sterilisant of rabies virus.(27) Vitamin C is included as the excipient during manufacture of BH4.

4. We have measured progressive lactic acidosis in CSF of 6 patients with rabies, associated with normal CSF glucose. Increased CSF pyruvate was measured in a single patient; lactate:pyruvate and lactate:glucose ratios were not classic for ischemia or mitochondrial disorders, although these were measured in CSF, not microdialysate.(28) The lactic acidosis was mild or reversible in a survivor and near-survivor.(29;30) See Figure.

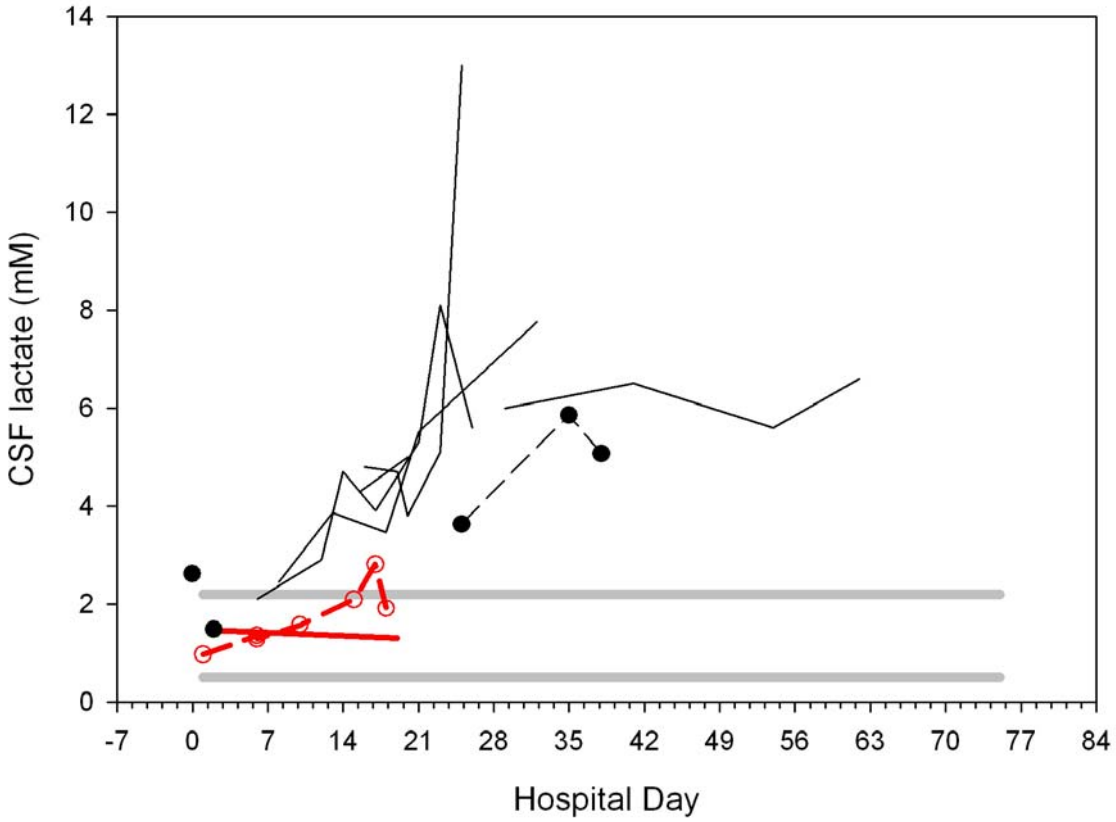

Figure: Progression of CSF lactic acidosis by hospital day by standard chemistry (lines) and by NMR (dots & dotted lines) in human rabies. In red are two survivors. Normal range is between the two grey lines.

5. We have measured early and persistent presence of elevated concentrations of quinolinic acid in the CSF of patients with rabies. The concentrations are more extreme than those encountered in cerebral malaria and HIV dementia, and in range for those encountered in bacterial meningitis. Quinolinic acid is a kynurenine metabolite of tryptophan, induced by interferon-gamma mediated inflammation. It is an NMDA glutamate receptor agonist and therefore an excitotoxin. Its activity is antagonized by kynurenic acid (low in rabies) and ketamine (NMDA receptor antagonist). See Figure:

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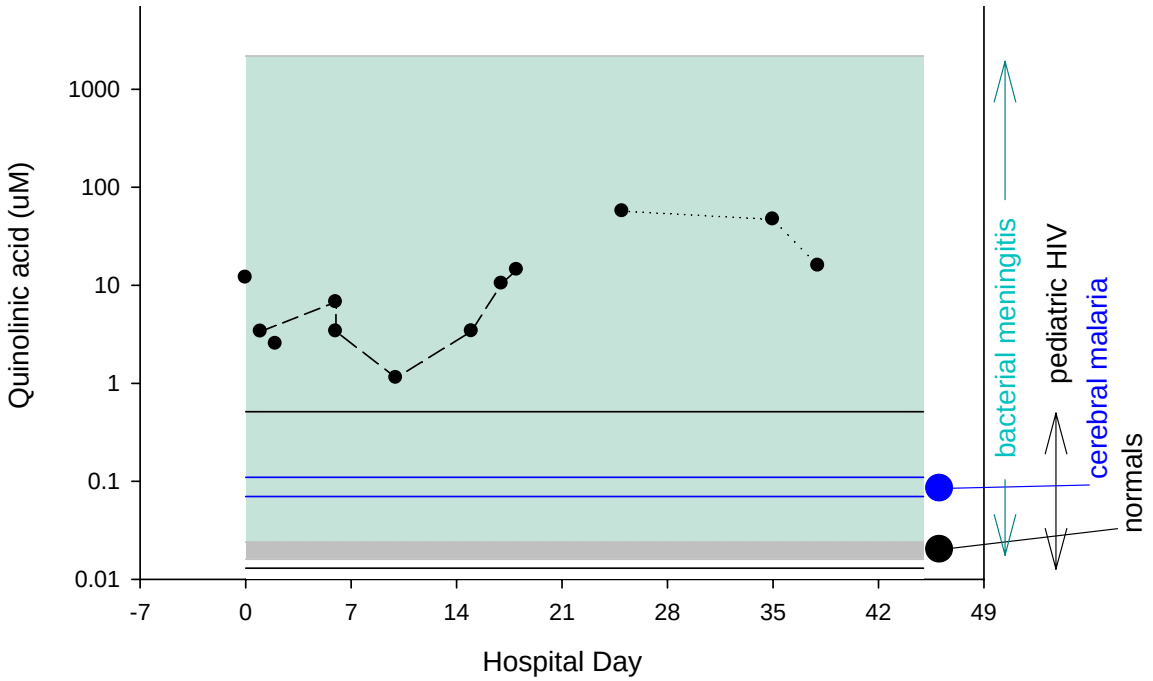

Figure: Concentrations of quinolinic acid by NMR in CSF from patients with rabies, by hospital day. Normal range is depicted by the gray band at the bottom. Range in cerebral malaria is depicted between blue lines. Range in pediatric HIV encephalopathy is below the black line. Range in bacterial meningitis is depicted by the light blue area.

MODIFIED PROTOCOL (version 3.1)

1. Patients should receive prophylactic supplementation with enteral vitamin C.
   Vitamin C 500 mg daily
   Our survivor also received zinc supplementation.
2. We no longer recommend replacement of BH4 if BH4 deficiency in CSF is proven acutely. *Survival from rabies has occurred in 3 patients NOT receiving continuous BH4 replacement therapy.(4;30)*
   - BH4 replacement at *low doses (2 mg/kg/day)* is recommended for systemic hypotension and with use of vasopressors.
   - BH4 replacement at *high doses (10-20 mg/kg/day*, divided Q12 h) is recommended during rehabilitation for 4-6 months when BH4 and/or neurotransmitter deficiency has been proven.

3. CSF should be assayed initially and then at least weekly for lactate, biopterin, neopterin, HVA and 5-HIAA. (See E.3. Biopterin deficiency in the CNS will reduce monoamine neurotransmitters.)

4. We recommend prophylaxis against vasospasm with nimodipine. Another calcium-channel blocker should be used if nimodipine is not available. *Use of nifedipine or magnesium sulfate in MP version 2 was associated with survival and near-survival, as well as avoidance of progressive CSF lactic acidosis*.(29) (See A.5. Evidence for metabolic deficiencies.)

5. We recommend ketamine and amantadine as putative neuroprotectants against quinolinic acid-mediated neurotoxicity, based upon our metabolic data.(31)

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6. Consider weekly monitoring for serum prolactin that may provide a useful index of hypothalamic integrity and brain dopamine metabolism.(20) Prolactin is used clinically in BH4 replacement therapy for malignant PKU and related pterin disorders.

A.6. Anticipation and prevention of cardiac arrhythmias and myopathy

Rationale

Early cardiac complications kill 20% of rabies patients. Most commonly, these are conduction disturbances ranging from asystole or bradycardia to supraventricular tachycardia. Asystole can occur without prodrome or closely follow tachycardia with hypertension.

- Rhythm disturbances abate with deeper sedation.(32;33)
- Clinicians noted that cardiac dysrhythmias were avoided by maintenance of normal oxygenation and oxygen carrying capacity (10 mg% of hemoglobin).(34) This recommendation is remarkably similar to recommendations for treatment of brain-dead organ donors-- rabies mimics many aspects of brain death. (35) Higher hemoglobin concentrations (12 mg%) are recommended during CNS resuscitation following head trauma in some centers.(36)

Note: we have seen loss of heart rate variability after 5-10 days of hospitalization. We infer that this is related to loss of autonomic innervation as predicted by anterograde transport of rabies virus through all peripheral nerves. Loss of autonomic variation was not related to induction of burst suppression.(4) Loss of nitrergic innervation reduces autonomic variability.(37)

Depressed ejection fraction is also reported as a late complication. This is speculated to involve infection of the myocardium, but the evidence to support this is not convincing in carefully done autopsy series.(38;39)

- Progressive hypotension may be due to loss of catecholamine synthesis in the adrenal medulla. The medulla is innervated and subject to rabies infection, possibly resulting in loss of BH4 and BH4-dependent catecholamine synthesis. *We have yet to measure catecholamines in blood or urine in this situation.* We have seen mild hypotension requiring vasopressors respond to 2 mg/kg/daily of BH4 supplementation on several occasions in early rabies.
- Progressive hypotension and cardiac dysfunction are attributed to hypothyroidism (low fT3) after brain death.(35) We have encountered hypothyroidism (low TSH, low T4, low T3, and low rT3) during week 3 of rabies with progressive bradycardia, hypothermia and hypotension.

ORIGINAL PROTOCOL

1. We recommend deep sedation-anesthesia during the first 7-10 days of acute encephalitis
2. We recommend red cell transfusion to maintain hemoglobin > 10 mg%, appropriate volume loading, and mechanical ventilation targeting arterial normoxia and mild hypercapnia.
3. Anticipatory placement of venous access for cardiac pacing wires is recommended.
4. Consider topical application of 1% lidocaine to the hypopharynx and trachea if reflex spasms or dysautonomia occur with care of the endotracheal tube

Caution: Isoproterenol used to treat severe bradycardia or conduction block may have unintended consequences by increasing cerebral blood flow and intracranial pressure. Judicious limitation of dose and duration are encouraged when it is needed.

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A.7. Lung function and ventilation

Rationale

Poorly understood abnormalities of oxygenation and lung function are frequently described in rabies.(34) The lung abnormalities appear consistent with transient pulmonary hypertension, if nNOS declined in rabies following acquired biopterin deficiency (see above A.5 Evidence of metabolic deficiencies). Approximately 30% of NO tone in the lung is contributed by nNOS innervation.(40)

1. Implementation of deep sedation-anesthesia requires ventilation of the patient.
   Intubation will likely be necessary for 2-3 weeks given the ontogeny of the immune response and rabies-associated paralysis.(4) (See G. Duration of care/Clearance of virus.)

2. Profuse salivation, from 1.5 to 6.0 L per day, is encountered at time of the acute encephalitis and recurs during convalescence.(4) Profuse salivation is a major risk for aspiration and may contribute to reflex spasms and dehydration.
   - Salivary secretions are quite viscid and can require bronchoalveolar lavage for effective clearance.
   - The salivary gland is the only organ where both sympathetic and parasympathetic stimulation lead to sialorrhea. We suspect that the stimulus is sympathetic and that anticholinergics may not work and may be contraindicated with risk of tachyarrhythmia.
   - Ketamine also induces salivation, but we have not generally seen any correlation of salivation with ketamine use in rabies.

3. Mild acidosis is considered to be neuroprotective by modulating glutamate receptor-mediated excitotoxicity in the brain.

ORIGINAL PROTOCOL

1. Patients should be intubated or undergo tracheotomy with cuffed tubes in order to protect the airway from aspiration secondary to profuse salivation, bulbar paresis, or rabies-associated or induced coma. *Note that cuffed endotracheal or tracheostomy tubes require verification of proper function and sealing pressure with every nursing shift.*
   Caution: Elective tracheotomy has been associated with subsequent neurological decompensation in two patients, when done during periods of high risk for vasospasm (days 6-14 of hospitalization) or with subacute cerebral edema. We suggest that such elective procedures are best done with exquisite anesthesiology at time of diagnosis or after the immune response is documented and subacute cerebral edema excluded (after 14 days).
2. We recommend red cell transfusion to maintain hemoglobin > 10 mg%, appropriate volume loading, and mechanical ventilation targeting arterial normoxia and mild hypercapnia.
3. Consider topical application of 1% lidocaine to the hypopharynx and trachea if reflex spasms or dysautonomia occur with care of the endotracheal tube.
4. The original survivor using the Milwaukee protocol developed reactive bronchospasm during her immediate convalescence that was responsive to albuterol nebulized treatments and adequate pulmonary toilet of her viscous secretions.(4)

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A.8. Temperature regulation and poikilothermia in rabies

Rationale

Rabies is a febrile illness during the prodrome and acute encephalitis phase. Rabies is later associated with poikilothermia in animals and man.(41;42)

In our index patient, fever abated during induction of therapeutic coma, only to recur at 12 days of hospitalization in association with development of neutralizing antibody in cerebrospinal fluid. There was mild hypernatremia (generally associated with fever) and increased T2 (FLAIR) signal by MRI, but minimal inflammation in spinal fluid (unpublished data, RW). The fever at time of immune response in our first patient was unresponsive to repeat induction of coma, antipyretics, and cooling blankets (that also vented warm air into the room). Fever dissipated swiftly with a change in ambient air temperature.(4)

Theoretical causes of fever during rabies may also include

- Deficiencies in antidiuretic hormone – an important negative-regulator of body temperature (see above A.3 Supportive care: Fluids, electrolytes, neuroendocrine).
- Serotonin syndrome, resulting from mismatches of dopamine and serotonin during acquired biopterin deficiency (see above A.5 Evidence of metabolic deficiencies).
- Uncoupling of oxidative phosphorylation. (See A.5 Preliminary evidence of metabolic deficiencies in cofactors involved in electron transport).
- Systemic or CNS inflammation (See A.11 Systemic inflammation in rabies).

Fever is generally considered beneficial during viral infections, but becomes ominous for encephalitis patients when above 40.5C.

ORIGINAL PROTOCOL:

1. Fever is tolerated below 39.0C without medication.
2. Antipyretics are administered for fevers > 38.9C. *Note that antipyretics were not clearly effective.*
3. Consider modifying ambient room temperature for severe hypothermia (<36.0C) or hyperthermia (>39.5C).

A.9. Generalized flaccid paralysis

Rationale

Rabies progresses to generalized paresis after the acute phase and as coma supervenes. This is usually evident by 10 days of illness. Paresis may include bulbar musculature and is frequently associated with disturbances of sphincter control of bowel or bladder.

Reports on the physiology of paresis include anterior horn cell disease or axonopathy as well as demyelinating disease physiologically similar to acute inflammatory demyelinating neuropathy (AIDP or Guillain-Barre-Landry disease).(43) An AIDP picture has been associated with administration of rabies-specific immune globulin to one patient.(44) Recovery of the first patient receiving the Milwaukee protocol, who was fully paretic when her sedation-anesthesia was waned, was rapid and anatomically inconsistent with demyelinating polyneuropathy.(4)

Deep vein thrombosis was a catastrophic event in one immobile patient who was otherwise surviving intensive care for rabies.(14)

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ORIGINAL PROTOCOL:

1. Heparin 10 U/kg/hour is administered as prophylaxis.
   Note: unfractionated heparin may be preferable to low molecular-weight heparin because it is reversible if a neurosurgical or other procedure is needed emergently.
2. Consider support hose or inflatable stockings as prophylaxis against deep vein thrombi.
   Note: we encountered increases in body temperature in association with support hose and intermittent-pressure boots in our patient.
3. Physical therapy should be regularly scheduled during the period of therapeutic coma and rabies-associated paresis to avoid joint contractures.
4. The patient should be frequently repositioned to avoid pressure ulcers.

A.10. Cerebral edema and brain perfusion pressure. Monitoring.

Rationale

Rabies infection is minimally cytopathic and inflammatory. Some groups have inserted intraventricular drains prophylactically to monitor intracranial perfusion, provide therapeutic drainage if needed, or instill medications. Intrathecal medications make little sense for a parenchymal brain infection.(11;45) Intrathecal administration of interferon-alpha at very high concentrations was ineffective in effecting a clinical response or clearing rabies virus from the brain.(46) Interferon-alpha is known to be toxic to the brain and brain cells; the type I interferon in the brain is interferon-beta.(47)

- *Placement of an intraventricular drain provides an opportunity for diagnostic brain biopsy when the burr hole is made.*
- *Near-infrared spectroscopy (NIRS) monitoring has NOT provided early warning of this complication.*
- *Transcranial doppler may detect progressive increases in resistive index with cerebral edema.*
- *It has been very difficult to obtain prompt neurosurgical intervention. It is strongly recommended that neurosurgery be consulted soon after diagnosis in order to familiarize the surgeons with recent advances in treatment of rabies, such that considerations of futility and fear of rabies can be addressed ahead of any emergent need for their services.*

1. Tetrahydrobiopterin (BH4) has been detected in 5 of 5 consecutive patients treated for rabies. (See A.5 *Evidence of metabolic deficiencies*.)

1. BH4 deficiency will lead to loss of nNOS activity with constriction of cranial arteries.
   - *TCD measures velocity, not flow. Interpretation of TCD results must be made in context of hematocrit, PaCO2, and cardiac output as well as many other factors.(48;49) For this reason, some experts require CT angiography to confirm TCD findings of vasospasm before medical intervention.*
   - *Increased* blood flow velocities (>400 cm/s) led to loss of electrical activity, followed by cerebral edema in the first week of therapy with the Milwaukee protocol in one patient.
   - Loss of blood-brain barrier function (as evidenced by marked increase in CSF protein), CSF lactic acidosis, and cerebral edema followed severe *reductions* of blood velocity detected by TCD. Reductions in blood flow velocity with high resistive (or pulsatility) index must be checked by emergency computed tomography with angiography to exclude cerebral edema and confirm vasospasm.
   - Cerebral edema with decreased blood flow by TCD is not likely to be vasogenic in origin. Hyperventilation in this setting may exacerbate the underlying pathophysiology by worsening

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vasoconstriction.(50) Hyperosmolar therapy and correction of underlying metabolic abnormalities is recommended. An intraventricular drain has proven useful acutely because edema is often transient and responsive to removal of ventricular CSF.(34)

2. Recently, a subacute form of cerebral edema has been seen after onset of the rabies-specific humoral immune response in serum in patients with bat rabies. One patient was receiving low-dose BH4 and low dose magnesium sulfate as prophylaxis against vasospasm; the second was receiving nimodipine prophylaxis against vasospasm. Cerebral edema was most prominent in the basal ganglia and thalamus and mesial temporal lobes, but eventually generalized to the cortices. (See Figure) Both patients had CSF titers of neutralizing antibody at least 1 log higher that the index survivor (> 10 IU by RFFIT assay), after removal of ribavirin from the protocol. Cerebral edema was also associated with hyponatremia (serum Na < 135 mEq/L) without decline in urinary output and high urinary sodium, suggestive of salt wasting.
    - The subacute cerebral edema lasted for 3 weeks, far beyond the natural history of cerebral edema associated with trauma, stroke, or asphyxia. There was no contrast enhancement by computed tomography. There was no clear antecedent asphyxia. We do not currently have CSF lactic acid measurements on these patients.

Note: We hypothesize that the edema is "cytomegalic"-- related to the presence of neutralizing antibody in the cytoplasm of neurons in a diffusely rabies-infected brain.(51) A similar clearance mechanism occurs in the Sindbis virus model.(52) Cytomegaly may also be related to the action of interferon-gamma in Sindbis-infected cells in tissue culture.(53) We have measured high concentrations of quinolinic acid in CSF in human rabies, which implies high brain levels of interferon-gamma.(54)

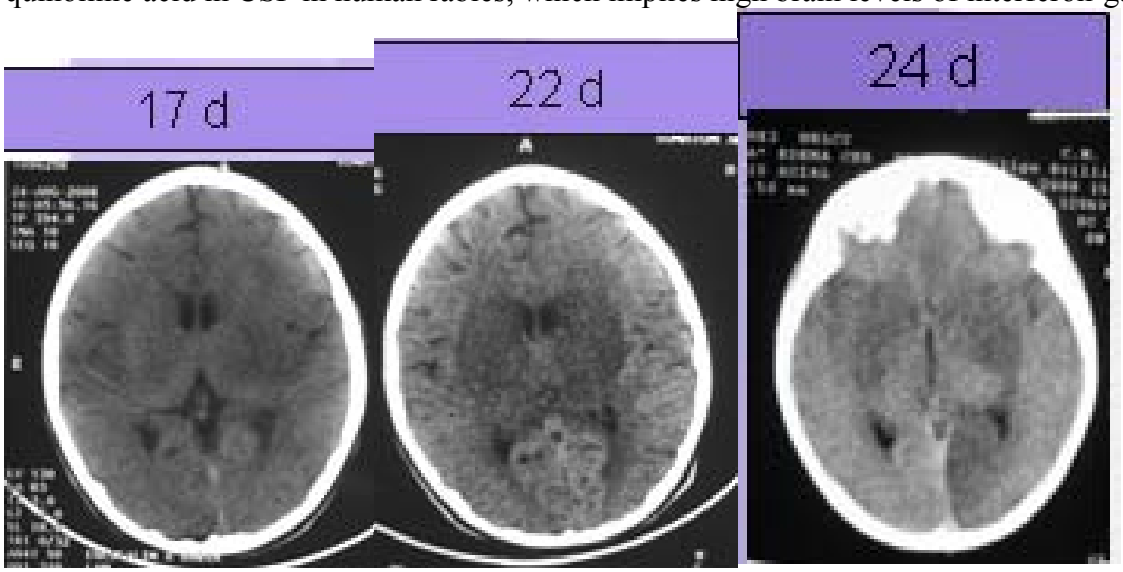

Figure: Progression of edema, initially in the basal ganglia with later cortical edema by CT. The edema was temporally associated with rapid increase in the rabies immune response. Despite severe edema of the basal ganglia (normally such edema would be catastrophic if cytopathic), the survivor had virtually normal basal ganglia by MRI after discharge from the ICU and, after demise from an unrelated event, had normal neuronal complement in the basal ganglia at autopsy.

MODIFIED PROTOCOL (version 3.1)

1. We recommend daily transcranial doppler (TCD) ultrasound monitoring of the middle cerebral arteries bilaterally for the first 2 weeks of hospitalization.

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2. Neurosurgical consultation is advised in anticipation of possible urgent need of an intraventricular catheter for monitoring and drainage of CSF.
3. No medications are given intrathecally.
4. Maintain serum Na concentration to at 140-150 mEq/L after advent of the serum antibody response to rabies for 7 days.
5. Consider computed tomography of the head 3-4 days after advent of neutralizing rabies antibody in serum to exclude incipient cerebral edema.
6. In bat rabies, if CSF neutralizing antibody is likely to pass > 10 IU, consider pulse corticosteroids to curtail the immune response. Preliminarily, doses of methylprednisolone of 30 mg/kg/day x 3 days or dexamethasone 1 mg/kg/day x 3 days fully suppressed further evolution of the immune response. Consider a shorter pulse.
    - Clearance of antibody from CSF is very slow, so titers > 3 are likely to persist indefinitely after a corticosteroid pulse.
    - In dog rabies, such cerebral edema has not yet been documented, and evolution of CSF immune response is slower.

A. 11 Systemic inflammation in rabies

Rationale:
While patients are often poikilothermic in rabies, they remain at risk for invasive bacterial and fungal infections while heavily sedated and/or paralyzed. Identification of infection in such patients is difficult.

Initially, rabies presents with either a normal or elevated white blood cell count, with a modest left shift. With the advent of a humoral immune response to rabies, rises in C-reactive protein and platelet counts (to greater than 1,000,000/mm^3) have been observed despite persistently negative evaluations for sepsis.

MODIFIED PROTOCOL (version 3.1)

1. Prophylactic use of antibiotics is discouraged. The course of treatment of rabies is long and such use will select for resistance.
2. Serial surveillance (gram stain + culture of endotracheal tube, blood and urine cultures) may be indicated given the poikilothermia and inflammatory overlap with bacterial sepsis.
3. Empirical use of anti-bacterial drugs, once initiated, should be limited to 3-5 days when all cultures remain negative.

A.12 Nutrition

Rationale
Rabies can be complicated by cerebral edema in association with hyponatremia from SIADH or salt wasting. In our experience, the nutritional demands of a medically sedated, often insensate and paralyzed (by rabies) patient on the ventilator are often overestimated. These over-estimates of the metabolic requirements in rabies result in administration of excess free water that often complicates urgent management of hyponatremia and cerebral edema.

1. The caloric requirements of a heavily sedated, immobile patient on the ventilator approximate the resting energy requirements (REE) for humans, as published by WHO. These vary by age and gender. There are various formulae for estimating REE. Online calculators are numerous.

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- For adults: http://www.bmi-calculator.net/bmr-calculator/metric-bmr-calculator.php
- For children: http://www.pediatriconcall.com/FORDOCTOR/pedcalc/basel_energy_expenditure.aspx
- Manual calculator, for adults:
  For men: BMR (REE) = 66 + 13.75(weight, kg) + 5.0(height, cm) - 6.76(age, y)
  For women: BMR (REE) = 655 + 9.56(weight) + 1.85(height) - 4.68(age)
- Manual calculator, for children:
  REE (MJ/d) = 0.02606 x weight (kg) + 0.04129 x height (cm) + 0.311 x sex (male, 1; female, 0) – 0.08369 x age (y) – 0.808

2. Enteral nutrition is best provided by nasojejunal tube, using "high salt", isotonic formulations.
   - These allow further sodium supplementation when needed. (See Appendix II, Salt wasting.)

3. Free water requirements are generally 100 ml of water per 100 kcal.

4. We recommend supplementation with vitamin C and zinc. Coenzyme Q10 supplementation is no longer recommended. BH4 supplementation is no longer recommended acutely, except in special circumstances. BH4 supplementation is essential during rehabilitation.

B. Neuroprotection and vasospasm

B.1. Neuroprotection

Rationale
A rabies vaccine-naive patient survived rabies when treated by a strategy that minimized dysautonomia.(4) Three of the Milwaukee protocol drugs (ketamine, midazolam, amantadine) are potentially protective of the brain and spinal cord through established pharmacologic mechanisms. While neuroprotection has rarely been shown to be effective in controlled clinical trials in human, virtually all such trials evaluated drugs singly and at doses insufficient to produce deep sedation. This contrasts with implementation of the original Milwaukee protocol.

1. **Ketamine** was chosen as the critical drug because of its properties as a dissociative anaesthetic (see above A.2 Supportive care: Palliation), an antiviral specific to rabies (see below C.2. Ketamine and amantadine), and its potential as a neuroprotective agent that non-competitively antagonizes excitotoxic N-methyl-D-aspartate (NMDA) glutamate receptors.(55)

   - There is often considerable reluctance to use ketamine because of beliefs that it is contraindicated in the setting of cerebral edema. This is not true – it is used for treatment of traumatic brain injury in Europe (see Appendix I-Annotated bibliography on ketamine).(56;57)

   - There are current FDA concerns that ketamine may lead to local neuronal apoptosis in select nuclei in neonates, based on animal models. Loss of brain volume in at risk areas was not noted in our survivor despite considerable ketamine exposure. Ketamine-associated apoptosis is minimized in animal models by concomitant use of GABA-enhancing sedatives.(58)

     From review of clinical literature, ratios of ketamine to midazolam in the range of 0.5-2.0 are optimal. From our experience, we recommend ketamine in doses of 1-2 mg/kg/h (achieved in first 24 hours) and midazolam in a ratio of ketamine: midazolam of 2:1 to 1:1.

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- The recent finding of the excitotoxin, quinolinic acid, in CSF in human rabies in 4 of 4 patients is an additional argument for use of ketamine in this clinical context. Ketamine inhibits quinolinic acid-mediated toxicity in animal models.(31)

2. **Amantadine**, like ketamine, is an NMDA antagonist that is also active against rabies virus *in vitro*.(59) Ketamine and amantadine bind differentially in rodent brains and were therefore presumed to be additive in their NMDA antagonism during rabies treatment.(4;59;60)
   - Memantine has not been tested against rabies virus.

3. **Midazolam** was used to balance ketamine, to further sedate the patient, lower metabolic demand, potentially block excitotoxic α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) glutamate receptors and stimulate protective γ-aminobutyric acid (GABA) receptors. Benzodiazepines are clearly inferior at inducing complete burst suppression – an advantage when the natural history of rabies is to lose EEG amplitude over 14 days.

   - Phenobarbital, another GABA agonist, was used in small doses (3 mg/kg) to repeat-induce burst suppression when large doses of benzodiazepine were insufficient for this purpose. This was encountered after 4-5 days of induced coma.
     Note: We do not recommend use of barbiturates until the immune response is well established. Barbiturates are potent immune suppressants (order: thiopental> pentothal> phenobarbital), primarily affecting lymphocyte proliferation at clinical doses, with induction of lymphocyte apoptosis at very high doses.(61) We have noted abnormal delays in appearance of rabies-specific antibodies in patients receiving multiple loading doses of barbiturates or with very high serum levels of phenobarbital. It may be that the main advantage of using ketamine for treatment of rabies lay in its regular complementation by benzodiazepines rather than barbiturates.
     See also D3. Ribavirin as Th1-skewing immunomodulator.

   - Propofol resulted in rapid and complete suppression of EEG activity in 2 rabies patients. Propofol is vasoconstrictive when used in animal models for the study of cerebral blood flow relative to some other anesthetics.(62) TCD was not being monitored during propofol sedation in these 2 patients. Propofol should be used with caution given BH4 deficiency and cerebral artery spasm recently described in rabies

MODIFIED PROTOCOL (version 3.1)

1. Ketamine is dosed at 1-2 mg/kg/h, achieved over the first 24 hours.
2. Midazolam is titrated in the range of 0.5 to 2.0 mg/kg/h (ratio of ketamine: midazolam from 2:1 to 1:1)
3. We no longer recommend use of barbiturates.
4. Amantadine is administered by nasojejunal tube at a dose of 2.5 mg/kg every 12 hours (100 mg every 12 hours if >40 kg).
5. We recommend *SLOW* discontinuation of sedation-anesthesia when (a) anti-rabies titers in CSF exceed 1:1024 by IFA or 1:89 by RFFIT (about 0.9 IU), or (b) there is evidence of denervation of the heart as shown by loss of heart rate and blood pressure variability (see A.6. Anticipation and prevention of cardiac arrhythmias and myopathy).(4) Ketamine may be tapered (every few hours) by 50% every 24 hours, to a basal rate of 0.5 mg/kg/h. Benzodiazepines may be tapered by 10% of the dose every day to every other day

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Key point 1: We no longer recommend titrating to burst suppression. The primary goal is avoidance of dysautonomia over the first 10 days of hospitalization.

Key point 2: Ketamine at doses ≥ 3.0 mg/kg/h was associated with progressive, reversible renal insufficiency in 2 patients treated at a single center. We discourage ketamine doses in this range or higher.

Key point 3: Benzyl alcohol, a preservative present in especially high concentrations in midazolam, may cause a metabolic acidosis (hippuric acidosis) with low serum lactate. The dosage of benzyl alcohol should not exceed 100 mg/kg daily. We encountered acidosis associated with a cumulative dosage of 362 mg/kg.(4)

Key point 4: Given the relatively uneventful course of rabies during coma therapy, we now recommend longer acting (and less toxic from less preservative) diazepam or alprazolam over midazolam. Midazolam 3 mg is equivalent to 1 mg of diazepam; half-lives are 2.5 h (midazolam) and 30-60 h (30-100 h for metabolite of diazepam)

- Diazepam is more lipid-soluble and may better control brainstem reflexes.
- Alprazolam is a "gold standard" PAF receptor antagonist. The fifth BH4-dependent enzyme is expected (but never proven) to affect PAF metabolism. (See A.5 Evidence of metabolic deficiencies)

Key point 5: Ketamine causes higher voltages on EEG, so that its discontinuation confounds interpretation of the predicted declines in EEG voltage from rabies or BH4 deficiency (see above *A.5 Evidence of metabolic deficiencies*).

Key point 6: Phenobarbital and pentobarbital appeared to control dysautonomia better than midazolam in one patient, perhaps because of their higher lipid solubility. More lipid soluble benzodiazepines are also available (diazepam).

Caution: Avoid using loading doses of phenobarbital. Loading doses were temporally associated with suppressed antibody response in one patient (with normal serum concentrations). Phenobarbital was administered originally in 3 mg/kg doses when needed.

B.2 Cerebral vasospasm in rabies

Rationale

Vasospasm was detected previous in rabies by angiography, MRA, HMPAO scan and transcranial doppler ultrasound.(14;63-65)

1. We have seen altered transcranial doppler (TCD) blood velocities in 3 of 4 cases where it was sought, but only at certain times. In a fourth patient who received experimental hyper-immunization, vasospasm was not detected before demise.

- We recommend use of TCD to detect this potential complication due to its simplicity and bedside availability.
- *Some experts recommend CT angiography to confirm vasospasm screened by TCD.*
- *Interpretation of TCD results must be made in context of hematocrit, PaCO2, and cardiac output as well as many other factors.(48;49)* (See E.2 Biopterin deficiency in the CNS may reduce cerebral perfusion)
- Near-infrared spectroscopy (NIRS) may not be useful in rabies. In one patient, when TCD studies indicated pronounced vasospasm in one patient, forehead NIRS did not change. However, a subtle drop in NIRS saturation was noted in our index patient on hospital day 9.

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2. By TCD, 2 forms of vasospasm have been observed:
   - Type 1 (conduit vessel spasm): High velocity blood in the middle cerebral artery bilaterally has been detected by TCD on **days 6-10 of hospitalization** in 2 patients. This is the usual time when clinical coma supervenes in rabies.
     - Resistive index was normal.
     - Peak blood velocities > 400 cm/s were associated with electrical seizure activity followed by EEG suppression in 1 patient.

   - Type 2 (distal arteriolar spasm?): Low velocity of blood flow has been seen bilaterally exactly 7 days after high velocity spasm, on **days 13-17 of hospitalization**.
     - We have not witnessed this later form of vasospasm except in patients who had clinical events in association with the time of type 1 vasospasm (not all patients were examined for vasospasm).
     - Resistive index or pulsatility index is markedly increased.
     - Despite high peripheral resistance that normally suggests cerebral edema, there was no cerebral edema radiologically.
     - Intracranial pressure is very low by ventriculostomy or lumbar puncture, suggesting loss of cerebral blood volume
     - Type 2 spasm has been associated with acute increases in CSF protein, as well as CSF lactic and pyruvic acidosis. Section A.5
     - At autopsy after type 2 spasm, there is no evidence for thrombosis, obliterative endarteritis or other process – only conventional venulitis consistent with encephalitis. nNOS enzyme is still evident by NADPH diaphorase staining.

   Caution: Low blood velocities by TCD may not be distinguished from normal in the elderly, given high variance of blood velocities reported in this age group.(66)

MODIFIED PROTOCOL (version 3.1)

1. We now recommend prophylaxis against vasospasm using nimodipine x 21 days.
2. Type 1 spasm responds to 2 mg/kg every 8 hours of BH4 orally or to *very low dose* nitroprusside (0.1-0.2 mcg/kg/min – this does not take much given hypersensitivity of NO-deficient tissue). (See also Section E.22 Biopterin deficiency in the CNS may reduce cerebral perfusion).
   - Nicardipine 75 mcg/min (pediatric dose: 0.5 mcg/kg/min), or "Triple H therapy", may make better sense.
3. Type 2 spasm may respond to 7 mg/kg every 8 h of oral BH4 (probably doses of 300 mg in adults) and 0.5 g/kg/24 h of IV L-arginine.
   - Nicardipine 75 mcg/min (pediatric dose: 0.5 mcg/kg/min), or "Triple H therapy", may make better sense.

C. Specific antiviral therapy against rabies

C.1. Ribavirin and vidarabine

Rationale

Ribavirin is a broad-spectrum antiviral recommended in the treatment of rabies.(9) It is active *in vitro* against rabies virus but does not cross the blood brain barrier well and has not worked in animals or humans.(10;67) Ribavirin DOES penetrate the BBB with chronic dosing.(68)

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Vidarabine has not been reported to be effective.(11)

Arguments in favor of use of ribavirin:

Ribavirin was used in the original Milwaukee protocol.(4) Our rationale for including ribavirin in the Milwaukee protocol, in light of its poor pharmacokinetics and efficacy against rabies, was to prophylax against myocardial infection by the rabies virus. We subsequently reviewed reports of rabies myocarditis and do not consider them convincing or an indication for use of ribavirin.(38;39) Ribavirin use was associated with pancreatitis and hemolysis at a cumulative dose of 276 mg/kg of ribavirin. Dosage was reduced one day early and discontinued one day early. (4)

Arguments against use of ribavirin:

A. Note: We have NOT seen a clear therapeutic effect by salivary PCR load or skin biopsy with the use of intravenous ribavirin, amantadine and ketamine in 3 patients receiving close approximations of the Milwaukee protocol.

B. Ribavirin was associated with delayed and depressed humoral immunity to rabies. (See D.3. Ribavirin as Th1-skewing immunomodulator and G. Duration of care/Clearance of virus).

C. Ribavirin is predicted to directly deplete BH4 (see above A.5 Evidence of metabolic deficiencies) by reducing guanosine pools that are precursors in the *de novo* synthesis of BH4.

- BH4 deficiency occurs in human rabies in the absence of use of ribavirin.

MODIFIED PROTOCOL (version 3.1)

1. We recommend that ribavirin NOT be used acutely.

2. There may be an indication for use of ribavirin for modulating vigorous immune responses that are recently associated with subacute cerebral edema. Similar arguments would apply to mycophenolate, a better known immunosuppressant that shares the same biochemical mechanism of action. For the moment, we are recommending a corticosteroid pulse as a more definitive intervention.

C.2. Ketamine and amantadine

Rationale

Ketamine and MK801 are NMDA antagonists with specific antiviral activity against rabies in animal models.(55;69)

Amantadine is an antiviral with indications for the treatment of influenza, and adjunctive use in Parkinson disease as an NMDA antagonist. Evidence for its activity against rabies virus is restricted to *in vitro* observations.(59)

Ketamine and amantadine bind differentially in rodent brains and were therefore presumed to be additive in neuroprotective effect during rabies treatment.(4;59;60) Ketamine and amantadine have no known, shared antiviral mechanism of action and should therefore be at least additive and possibly synergistic.

ORIGINAL PROTOCOL

1. See B. Neuroprotection and . for ketamine dosing and balancing with a *benzodiazepine*

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2. Amantadine is administered by nasojejunal tube at a dose of 2.5 mg/kg every 12 hours (100 mg every 12 hours if >40 kg).
3. We recommend gradual discontinuation of ketamine when (a) anti-rabies titers in CSF exceed 1:1024 by IFA or 1:89 by RFFIT (about 0.9 IU), or (b) there is evidence for denervation of the heart. Amantadine may be continued for an additional 1-2 weeks.

C.3. Interferon-alpha

Interferon-alpha (IFNα) shows potential for prophylaxis in animal models but was ineffective in treating human rabies.(10) There are numerous CNS toxicities associated with IFNα therapy, including psychosis and spastic diplegia, as well as the potential to deleteriously affect brain histology in rabies.(11;46)

IFNα administration suppresses antibody production that is theoretically necessary to clear rabies virus.(46)

Original Protocol
We deliberately avoid systemic or intrathecal administration of IFNα.

D. Immune modulation

D.1. Primacy of antibody response in clearance of rabies

Rationale
Autopsy reports of patients surviving for several weeks document clearance of the rabies virus.(13;14) Clearance appears to involve primarily the humoral arm of the immune system, particularly CD4 cells and antibody.(70) All 6 known survivors showed remarkably rapid rise to high concentrations of total and neutralizing rabies-specific antibody.(6)

Because immunoglobulins do not normally cross an intact blood-brain barrier (BBB), it is not clear that passive immunotherapy, administered systemically, enters the CNS.

Administration of antibody into ventricular or lumbar CSF is of questionable efficacy for a parenchymal disease such as encephalitis.(11;45)

Conversion of encephalitic to paralytic rabies was reported in a single patient following passive immunotherapy.(44)

In an animal model of Sindbis (alphavirus) encephalitis, similarly cleared by antibody without sequelae, antibody clearance of brain infection involves internalized antibody that inhibits transcription or translation. Virological clearance takes from 6-20 days after passive transfer of hyperimmune serum and 2 days after administration of monoclonal antibodies.(52)

We expect clinical improvement to occur subsequent to clearance of salivary viral load by PCR and before full virological clearance of rabies virus from the brain. This expectation is supported by a recent autopsy case showing irregular, heterogeneous presence of rabies virus by genome amplification (PCR) and antigen detection (DFA) without cultivation of infectious virus in a patient who was clinically improving at time of demise.

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MODIFIED PROTOCOL (version 3.1)

1. We recommend AGAINST administration of rabies-specific immune globulin systemically to immune-competent patients after onset of symptoms.
2. If the patient acquired rabies through transplantation with significant immunosuppression, then we recommend
    1. Consider discontinuation of all immunosuppressive drugs.
    *Mycophenolate is associated with depletion of biopterin through the same mechanism as ribavirin (IMPDH inhibitor).*Consider assay of CSF biopterin and neurotransmitter concentrations if patient received mycophenolate.
3. Do NOT use ribavirin (see D.3. Ribavirin as Th1-skewing immunomodulator and A.5 Evidence of metabolic deficiencies)
4. Avoid barbiturates.(see B.1 Neuroprotection)

D.2. Avoid vaccination while symptomatic, including accelerated vaccination

Rationale

Administration of an inactivated vaccine may theoretically skew the natural immune response by altering the distribution of responsive lymphocyte types and dominant epitopes.(71) Intramuscular administration of rabies vaccine during the acute phase is without apparent benefit.(9) Anecdotal case reports and animal studies suggest that this may accelerate and exacerbate the disease process.(72) There are alterations in the blood brain barrier of rabies patients previously exposed to rabies vaccine.(73)

- We registered 2 patients receiving rabies immunization after onset of symptoms who rapidly died.

- We registered 1 patient who developed rabies while receiving the IM series of cell-culture derived vaccine without rabies immune globulin. That patient survived, but with MRI evidence of white matter damage that is atypical of wildtype rabies infection. The neurological recovery of that patient is uncertain, but may involve paresis and/or spasticity. This patient, as well as 5 antecedent survivors of rabies after failure of vaccine-only prophylaxis, indicates that survival is possible under this scenario but that neurological outcome is guarded.

- Accelerated administration schedules by multiple-site intradermal (ID) immunization for treatment of rabies may result in an immune response in less than 7 days, a theoretical benefit when rabies is often fatal in 5-7 days without intensive care. Median survival with intensive care is 18 days. Therapeutic efficacy of an accelerated ID schedule is not known. It is unclear why this approach should benefit the patient when administration by the IM route does not and is potentially harmful.

ORIGINAL PROTOCOL

We do NOT recommend immunization of the patient after onset of clinical symptoms.

D.3. Ribavirin as Th1-skewing immunomodulator

Rationale

Ribavirin is capable of skewing the immune response toward an inflammatory, Th1 phenotype. This effect is mediated by modulation of cytokines as well as selective toxicities to different lymphocyte lineages and is independent of its antiviral effect.(74-76)

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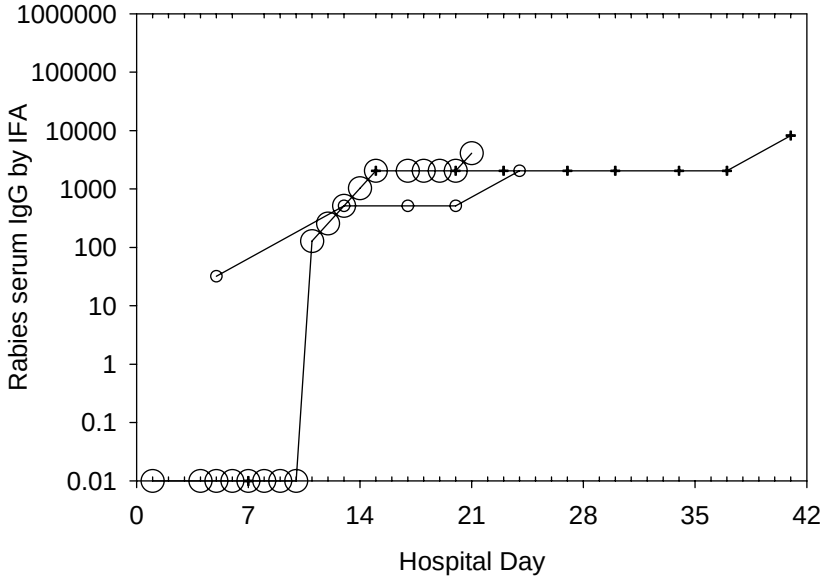
Given the primacy of the humoral immunity (Th2 response) in clearance of rabies, use of ribavirin as an antiviral must be weighed against its immunomodulatory properties.

- Ribavirin suppresses or delays antibody production in primates infected with Lassa virus. The effect persists for 5-7 days after cessation of dosing.(77) (Figure, below.)
- The same phenomena have been recorded in human rabies. (Figure, below.)
- Rabies neutralizing antibody titers in patients receiving ribavirin are below the median for patients with both dog- and bat-associated rabies. (Figure, below)



Antibody responses in 3 rabies patients over the first 42 days showing peculiar plateaus in antibody responses lasting for weeks.

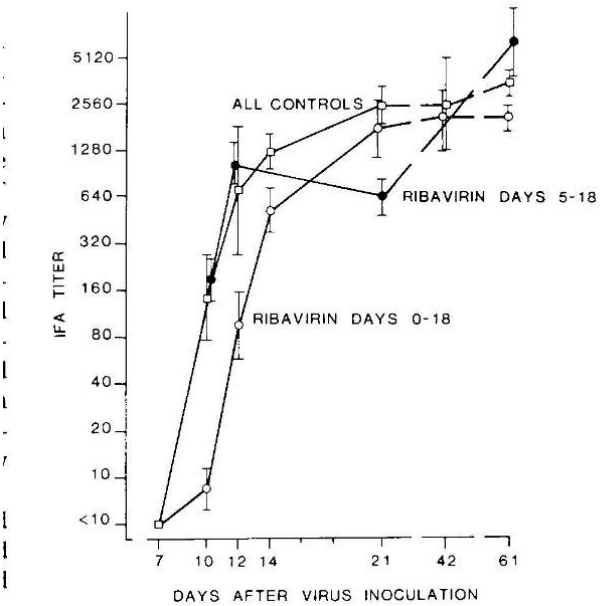


Figure from **Jahrling PB (1980) J Infect Dis 141: 580** showing same phenomena in macaque monkeys with Lassa virus, treated with ribavirin.

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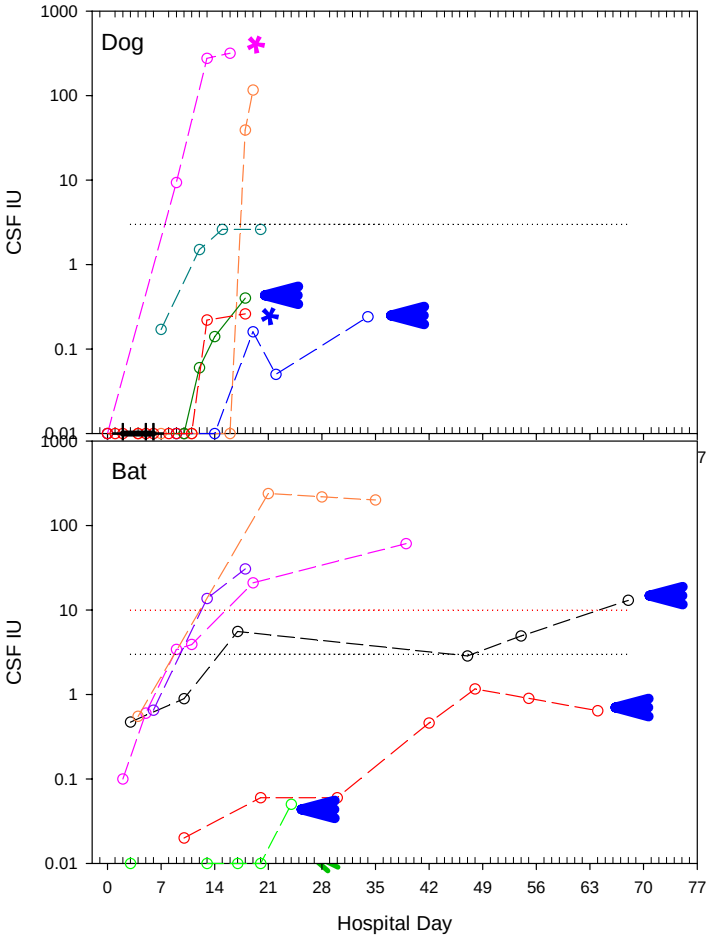

Figure: Rabies virus neutralizing antibody responses in CSF (RFFIT assay) by hospital day in patients with dog (upper panel) and bat-associated rabies (lower panel). Patients receiving ribavirin are identified by blue arrows. Pink asterix: patient experimentally hyperimmunized. Blue asterix: patient who received corticosteroid pulse empirically after high serum antibody was detected.

D.4. Passive immunotherapy

Rabies-specific antibody is barely detectable in serum after standard dosing of rabies immune globulin (RIG). For this reason, some experts even question the wisdom of administration of RIG other than into the wound during post-exposure prophylaxis.

Rabies is almost exclusively neurotropic and systemic antibody is unlikely to be efficacious once virus enters the nervous system. Immunoglobulins (and interferons) do not normally cross an intact blood-brain barrier (BBB). It is therefore not clear that passive immunotherapy, administered systemically, would enter the CNS.(9)

Administration of antibody or drug into ventricular or lumbar CSF is of questionable efficacy for a parenchymal disease such as encephalitis. (11;45) Intrathecal administration of interferon-alpha at very high concentrations was ineffective in effecting a clinical response or clearing rabies virus from the brain.(46)

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Administration of large amounts of RIG converted one "furious" rabies patient into the polio-like "paralytic" form, with clear histologic damage to the spinal cord.(44) Similar passive immunotherapy with rabies immune globulin into irradiated mice, infected with a highly attenuated virus, caused the "early death" phenomenon. Neurological demise in mice included convulsions and tonic spasms atypical for rabies in this model, and was not associated with cytopathic changes to the brain.(78)

D.5. Subacute cerebral edema associated with vigorous immune responses in CSF

Ribavirin was discouraged in MP version 2 to avoid immunosuppression. We then had 2 consecutive patients who developed subacute-onset cerebral edema. Both patients were receiving modest doses of different vasodilatory agents (nimodipine, BH4 and magnesium sulfate) as prophylaxis against generalized cerebral artery spasm. Cerebral edema closely followed advent of humoral immunity to rabies in CSF, and severity of edema progressed as CSF titers increased. Both patients developed CSF titers at least ten-fold higher than those in the index rabies survivor. (Figure, below).

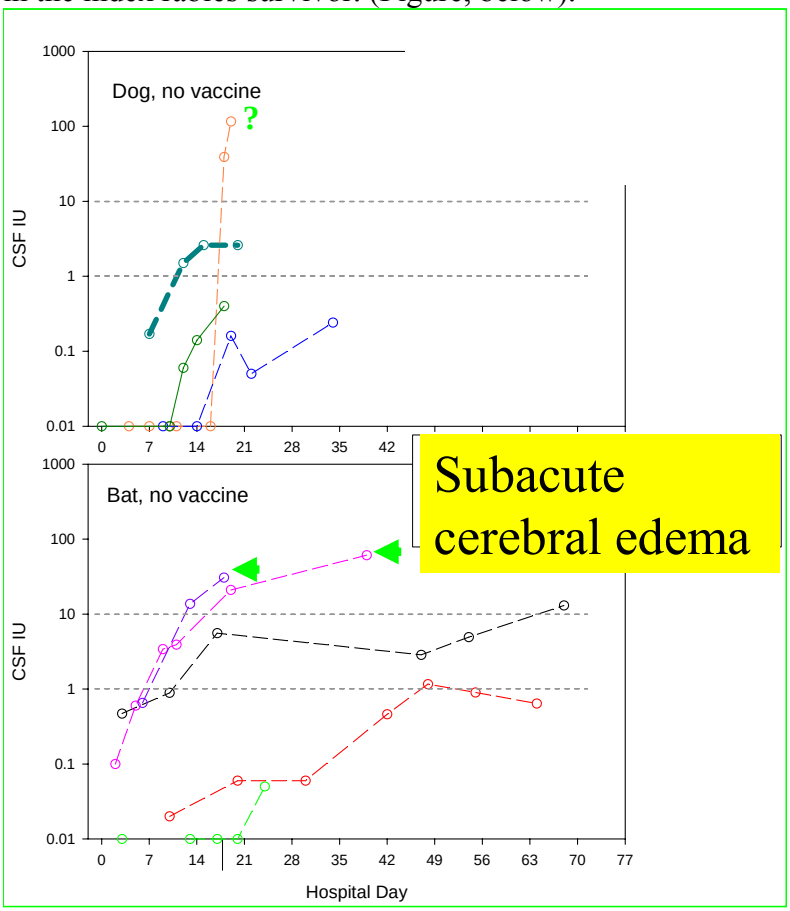

Figure: Rabies neutralizing antibody in CSF for rabies patients who did not receive rabies vaccine, by hospital day. Dashed grey lines, denoting 1 IU and 10 IU, include the range of CSF titers (dashed black lines) associated with clinical neurological recovery of the index survivor, who received ribavirin. Two subsequent patients with bat rabies who did not receive ribavirin (green arrows) produced titers at least a log higher than the index survivor and both developed cerebral edema-- fatal in one. A single dog-associated patient also developed titers > 10 IU. That patient improved neurologically but succumbed to renal failure.(29) *It is not yet clear whether cerebral edema is associated with high CSF rabies antibody titers in dog-associated rabies.*

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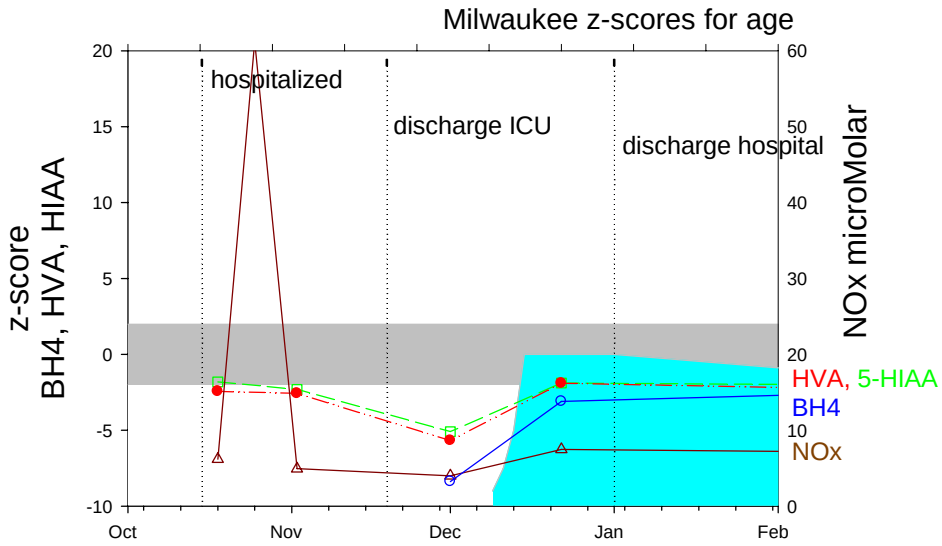
E. Biopterin deficiency in rabies

E.1. Evidence for biopterin deficiency in rabies

Deficiencies in tetrahydrobiopterin (BH4) in the lumbar cerebrospinal fluid (CSF) are now reported in 5 of 5 consecutive patients with rabies treated with the Milwaukee protocol.(5) Timing of the deficiency is unclear, but occurred within at least 1-2 weeks of onset of illness. In our survivor, BH4 deficiency was still evident in the second month when untreated. We also measured lower-than-predicted concentrations of BH4 in ventricular CSF – there are no established normal values for ventricular CSF – in an additional patient with rabies.



Our index survivor's course. Y-axis is z-score for age for BH4, HVA (dopamine metabolite) and 5-HIAA (serotonin metabolite). NO metabolites are absolute values (micromolar). Turquoise blue indicates BH4 supplementation during convalescence, resulting in higher concentrations of BH4, neurotransmitter metabolites, and NO metabolites.

CSF pterins include

- 7,8-dihydro-neopterin ("neopterin")
- 5,6,7,8-tetrahydrobiopterin ("BH4", reduced biopterin essential for monoamine neurotransmitter synthesis and for coupling or synthesis of nitric oxide synthases)
- Quinonoid BH2 ("q-BH2", oxidized form recycled back to BH4, that tautomerizes into 7,8-BH2).
- 7,8-dihydrobiopterin ("BH2", oxidized form inhibitory for NOS and incapable of efficient recycling to BH4 in the brain)

BH4 is oxidized by light and oxygen at physiologic pH into a variety of end-products that cannot be quantitatively detected. **BH4 must be collected into tubes containing antioxidants and immediately frozen on dry ice.**

- CSF for pterins is best collected into a tube containing 1 mg/ml (final volume) of dithioerythritol (DTE) and 1 mg/ml of diethylenetriaminepentaacetic acid (DETAPAC).
- Ascorbate, 1 mg/mL final volume, is a less efficient alternative.
- Flash freezing at bedside without preservative may yield useful samples.

Key point: There is a rostro-caudal gradient of pterins by lumbar puncture. The first 0.5 mL of CSF should be used for neurotransmitters, the second 0.5 mL as reserve, the third 0.5 mL collected with anti-oxidants and

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immediately frozen for assay of pterins, and additional CSF then collected for other studies. All should be immediately frozen.

There are several excellent laboratories capable of assaying for BH4 and neurotransmitter metabolites. These include:

Keith Hyland (Atlanta, GA): www.medicalneurogenetics.com
Georg Hoffmann (Heidelberg, Germany): Georg.Hoffmann@med.uni-heidelberg.de
Note: while not certified for clinical assays, we can provide rapid turn-around for iterative intensive sampling if needed for a rabies patient. rabies@chw.org

E.2. Biopterin deficiency in the CNS may reduce cerebral perfusion

Rationale:
BH4 is required at nanomolar concentrations for the proper synthesis of all isoforms of nitric oxide synthase (NOS). Proper coupling of BH4 also depends on the ratio of reduced BH4 to its irreversibly (in the brain) oxidized metabolite, 7,8-BH2.(23)

1. Rabies is almost exclusively neurotropic, and so should almost exclusively affect neuronal NOS (nNOS). Nitrergic (NO-containing) neurons innervate the adventitia of most vessels down to the terminal arterioles.

- nNOS contributes approximately 10% of systemic but 50% of cerebral blood flow (CBF).(79) This is the lower limit for pressure autoregulation of CBF.
- With loss of NOS activity, use of vasopressors reduces blood flow to 10%.(80) Nitrergic innervation of the anterior cerebral circulation proceeds through the geniculate and pterygopalatine ganglia, and includes the internal carotid artery extracranially.

  Key Point: Vasospasm is generalized in rabies.
  Key Point: MCA: ICA ratios are not informative in rabies because of anatomic innervation.

- We encountered ischemic damage to the medulla (posterior circulation) associated with abnormally low but present velocities in the MCA and ICA in one patient. This may reflect different nitrergic innervation of the anterior and posterior circulations.(81)
  Key point: Anterior cerebral circulation, including internal carotid artery, is innervated by nitrergic neurons via trigeminal and pterygopalatine ganglia.(79;81) Bites to the face may knock out cranial artery nNOS during retrograde transport to the CNS, before clinical signs appear, placing this group at higher risk.

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2. As indicated under Theory, there are 2 forms of vasospasm identified by daily TCD (Table and Figure).

type	MCA velocity	Resistive index	Onset (Hospital day)	Effective treatments by TCD
1 (conduit)	Increases until critical value (>350 cm/s), then falls precipitously	Normal	6-10	1. Nimodipine prophylaxis is recommended. Adults: 60 mg PO Q4h x 21 days. [This is about 1.5 mg/kg/dose in children.] IV formulation (adults and children): start 7.5-10 mcg/kg/h initially, increasing over a few hours to 30 mcg/kg/h (max 45 mcg/kg/h). 2. BH4 at 2 mg/kg Q8-12h (adult: 80 mg Q 8-12h) -or- Low-dose nitroprusside 0.1-0.2 mcg/kg/min. Caution: Nitroprusside may increase brain blood volume. Monitoring of intracranial pressure recommended.
2 (distal)	Decreases	Increases to 0.9	7 days after type 1 (13-17)	1. Nimodipine prophylaxis is recommended. 2. BH4 at 7 mg/kg Q8-12 (adult 250 mg Q8-12h) -plus- L-arginine 0.5g of 10% IV formulation over 24h 3. Consider IV nicardipine 75 mcg/min (pediatric dose: 0.5 mcg/kg/min)

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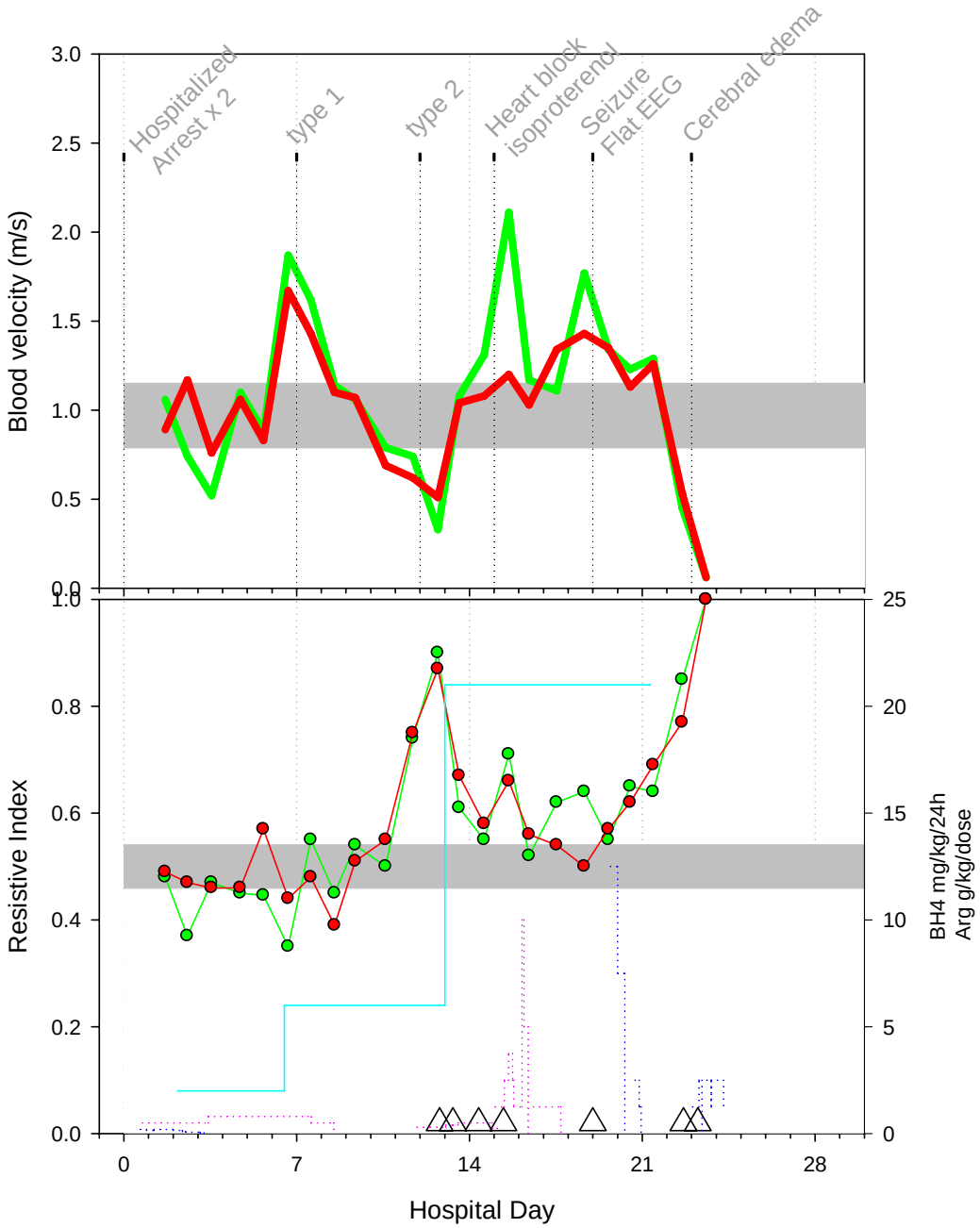

Figure: Daily trans-cranial doppler ultrasounds on a 10 year old with rabies. Rabies type 1 spasm on hospital day (HD) 6 is inferred from increased blood velocities in middle cerebral arteries (Upper panel, red=left, green=right, normal range shown as grey band) with normal resistive indices (Lower panel). Type 1 vasospasm responded to BH4 administered at 2 mg/kg every 8 hours (Lower panel, blue line). Rabies type 2 spasm on HD 13 is associated with low blood velocities (upper panel) and high resistive indices (Lower panel). This responded to BH4 administered at 7 mg/kg every 8 hours and infusions of 0.5 g/kg L-arginine (Lower panel, blue line and black triangles, respectively). (3)

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3. Prophylaxis appears more effective than therapy.
   - Nimodipine (82;83) likely acts as neuroprotectant rather than vasodilator.
   - Nifedipine was used with early success in a patient in Equatorial Guinea. The enteral dose of 0.1 mg/kg was occasionally too potent. It is probably safer to titer the dose using sublingual administration. Significant effect is apparent within 15 minutes, with maximal effect and tachycardia within 1 hour. Duration of effect exceeds 6 hours without high renin states. We would expect a dose in the range of 0.01 to 0.04 mg/kg/dose to cause mild tachycardia without effect on blood pressure.(84)
   - nNOS is present histologically at autopsy after type 2 spasm, so stabilizing nNOS remains a practical alternative.
   - Magnesium sulfate at a dose of 50 mg/kg IM was used in preference to calcium-channel blockers in a recent survivor. Vasospasm was not ascertained.
   - The precise 7-day interval between type 1 and type 2 vasospasm suggests that type 2 might require induction of genes following type 1 spasm.
     - "Statins"(85;86) increase BH4 and also prevent "microvascular rarefaction", an induced response that may explain the high vascular resistance and lactic acidosis encountered in type 2 vasospasm.
4. Treatment of vasospasm
   - Nicardipine is more effective as cerebral vasodilator than nimodipine.(87) Induced vasodilatation makes sense for confirmed vasospasm, but risks vasogenic cerebral edema.
   - Nitropaste is also effective for some forms of vasospasm(88)

Key point: *It is not yet clear how to best treat cranial artery spasm in rabies.*

5. NOS isoforms

   Paradoxically, in animal models, while high levels of NOS activity during acute traumatic brain injury or stroke are associated with more deleterious outcomes, NOS activity is required in later weeks for brain recovery. In humans, there is relatively little iNOS induction, such that the acute increase in NOS is found in the cerebral vasculature rather than brain.(89)
     - Delivery of "vascular doses" of BH4 should address primarily vascular disease.
     - We only recommend "CNS doses" of BH4 during rehabilitation, after confirmation of BH4 deficiency.

   CSF nitrate concentrations reflect primarily BBB function and so are not informative.(90;91)

E.3. Biopterin deficiency in the CNS will reduce monoamine neurotransmitter metabolites

BH4 is necessary for synthesis of catecholamine and serotonergic neurotransmitters. Low CSF concentrations of BH4 were found in human rabies along with low concentrations of the dopamine metabolite homovanillic acid (HVA) and the serotonin metabolite 5-hydroxyindoleacetic acid (5HIAA).(5)

- In one patient, BH4 deficiency preceded a precipitous decline in HVA. When concentrations of HVA in the CSF became undetectable, the EEG voltage diminished but brain integrity and blood flow, assessed by magnetic resonance imaging and transcranial Doppler ultrasound, were preserved. (See Figure.) One interpretation is that diminished or flat voltage by EEG (expected by day 14 of rabies) is a consequence of catecholamine neurotransmitter deficiency rather than

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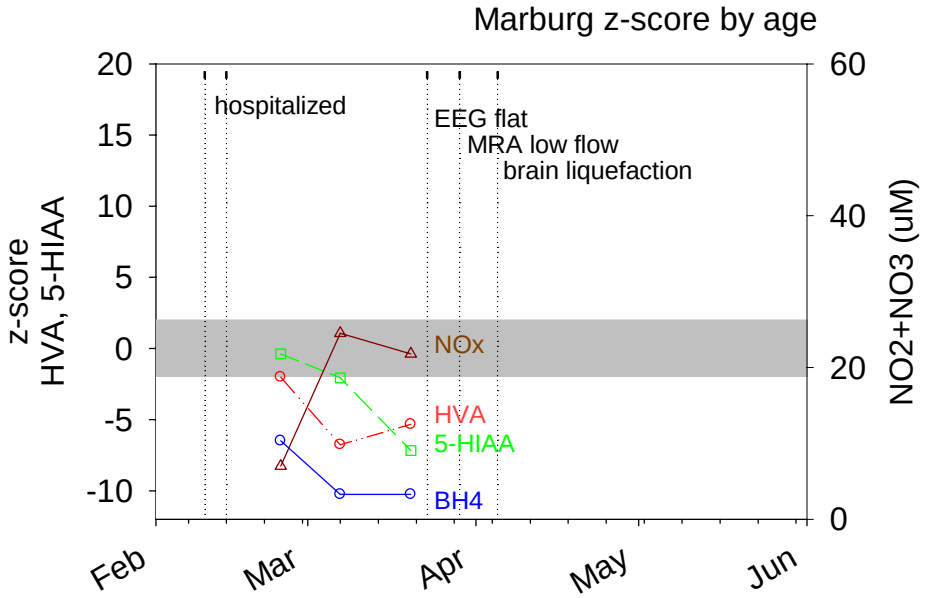
brain death. In cats depleted of catecholamines over several days using multiple pharmacologic interventions, the EEG declines.(92;93)

- Two patients with decline in EEG had associated cranial artery spasm. Spasm responded to NO-donor drugs or BH4 and L-arginine.
- In three patients, BH4 supplementation at 20 mg/kg/day resulted in extremely fast clinical improvement along with normalization of HVA and 5-HIAA neurotransmitter metabolites.(5)



Transplant recipient initially tested for BH4 deficiency just before decrease in EEG voltage. BH4 was not detectable. Retrospective assay of frozen CSF shows deficit 2 weeks after hospitalization. Flat EEG was associated with relatively normal MRI imaging and TCD studies. One week later, MRA and TCD showed decreased blood flow to the brain. Cerebral edema developed one week after that.

Note: Patients with congenital metabolic disorders causing BH4 deficiency vary in their response to BH4 supplementation, so metabolic precursors to dopamine and serotonin – past the congenital block – are often used adjunctively. Supplementation consists of L-DOPA with/without hydroxytryptophan plus an MAO inhibitor (carbidopa or benserazide). *We have no experience with neurotransmitter replacement therapy using L-DOPA and carbidopa,* although dosing in congenital deficiencies starts at 10% of the usual maintenance dose with gradual increments given hypersensitivity of such patients to the monoamine neurotransmitters.(94) Parkinson disease drugs such as Sinemet (L-DOPA/carbidopa) and Madopar (L-DOPA/benserazide) might be considered as cheap, ubiquitous substitutes until BH4 can be obtained. Ropinirole, a dopamine agonist, is another option.

Key point: There is a rostro-caudal gradient of pterins by lumbar puncture.

E.4. Biopterin deficiency may cause hypotension

BH4 is found in high abundance in the liver (basis for PKU), brain, adrenals, bone marrow and spleen, and small intestine.(95) The adrenal medulla is innervated and the neurotropic rabies virus has been localized to the adrenal, where it might deplete adrenal BH4 and BH4-dependent catecholamines. Insufficiency of the adrenal medulla should lead to hypotension. *There have been no samples of blood or urinary catecholamines in patients*

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with hypotension prior to use of vasopressor support, so there is no proof that this occurs. On the other hand, 2 rabies patients requiring vasopressor support initially had their support rapidly weaned off once low doses of BH4 supplementation (≥2 mg/kg/daily) were instituted.

ACTH stimulation test was abnormal in one patient on day 5 of hospitalization, after cardiac arrest.(14)

E.5. Cardiopulmonary effect of biopterin deficiency

nNOS contributes 30% of vasodilator tone to the pulmonary circulation, an effect much greater than the 10% contribution to the systemic circulation.(96;97) Partial NO deficiency in the pulmonary circulation is clinically mild unless the patient is stressed by hypoxia.(98) Of note, transient episodes of hypoxia without clearly defined mechanism are regularly described in rabies.(14;34)

Increased CVP has also been noted regularly in rabies despite moderately good function assessed by cardiac echocardiography.

G. Expected Duration of Care (Viral clearance)

Rationale

The Milwaukee protocol is based on two assumptions:

- Rabies virus infection (wildtype strains) is not cytolytic and poorly inflammatory. Death is primarily attributable to reversible dysfunction rather than irreversible destruction of brain, spinal cord and nerves. This can be suppressed temporarily by aggressive sedation.

- Survivors of rabies through intensive medical care rarely have virus detected 2 weeks after onset of symptoms, although residual virus can be detected in brain for an additional 1-2 weeks. The natural immune response is evident by the second week of illness and is sufficient to clear the virus.

G.1 Serology and recovery from rabies

Correlates of survival include detectable serum or CSF rabies-specific antibodies at time of diagnosis.(6) . Inclusion or exclusion criteria based on presence of antibody at time of diagnosis are arbitrary because antibody response is brisk with normal immunity and appears from one day to the next in serum. (See figure).

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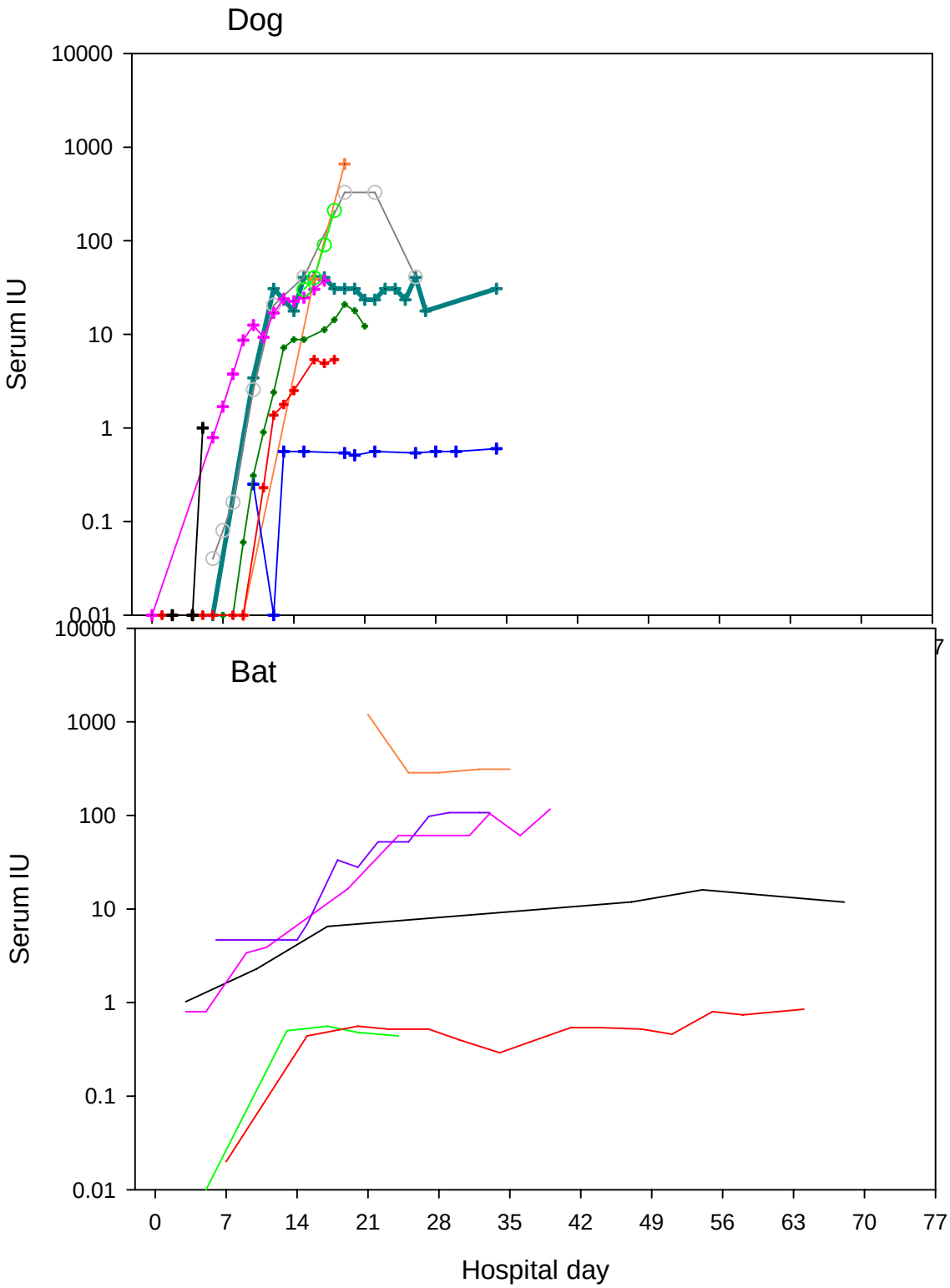

Figure: Kinetics of serum neutralizing antibody to dog and bat-associated rabies, by hospital day. Note that detectable serum neutralizing antibodies may be detectable earlier in bat associated rabies, and that the velocity of rise of titers in serum in dog-associated rabies is greater.

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Recovery of neurological function was closely associated in time with the rapid increase in CSF neutralizing antibody. (See Figure.)

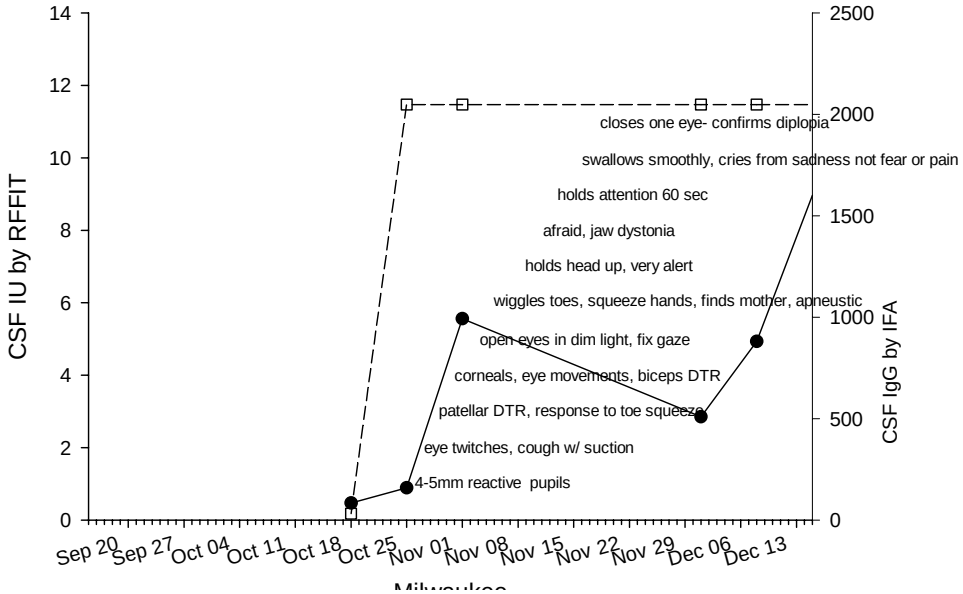

Figure showing Total rabies specific (dotted line) and neutralizing rabies antibody in cerebrospinal fluid relative to recovery of neurological function in our survivor.

For the first survivor of rabies receiving the Milwaukee protocol, antiviral therapy consisting of ketamine, amantadine and ribavirin was discontinued when the following titers were reached:

- CSF IgG by IFA > 1: 1024
- serum IgG by IFA > 1: 512
- CSF neutralizing antibody by RFFIT = 89 (about 0.9 IU)
- serum neutralizing antibody by RFFIT = 229 (about 2.3 IU)

G.2 Salivary viral load and recovery from rabies

Treatment with IV ribavirin, ketamine, and oral amantadine was NOT associated with a decline in rabies amplicon titer (viral load) by PCR in saliva. On the other hand, there has been a clear decline in salivary viral load after the advent of a systemic rabies-specific antibody response. (See Figure.)

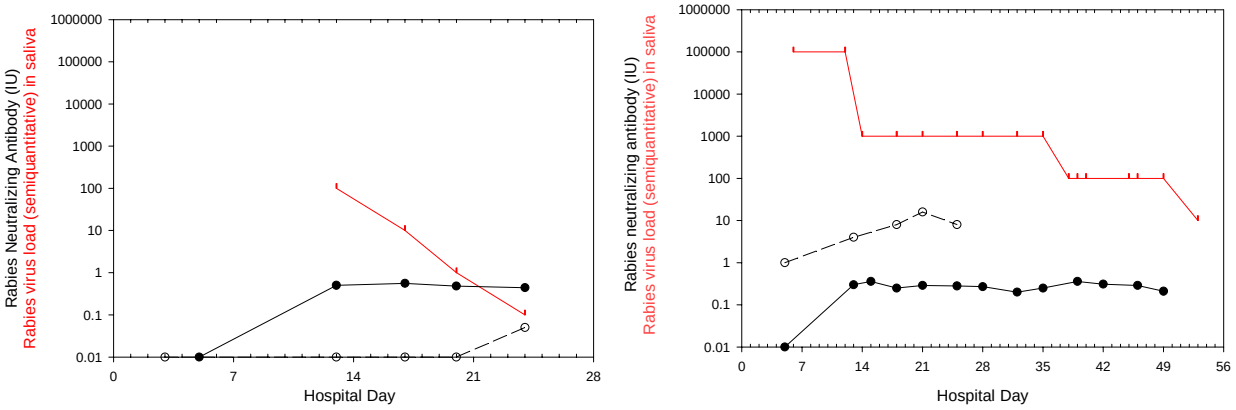

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Figure: Decline in salivary virus load (red, by semiquantitative PCR) in 2 patients in association with serum (solid circles) and CSF (open circles) neutralizing antibody (IU, by RFFIT) by hospital day. Note that decline is associated with modest neutralizing antibody in serum, but not necessarily CSF antibody.

G.3 Discontinuation of isolation

Rabies virus load, previously detected by PCR, was eliminated from saliva or skin in two recent survivors who developed serum and CSF neutralizing antibodies. Transmission of rabies is contingent on virus in saliva and tears, so the absence of viral genome in these fluids (for an amplified virus isolate) in the presence of > 3.0 IU is strong evidence for the clearance of rabies virus from saliva and the absence of transmissibility.

MODIFIED PROTOCOL (version 3.1)

A patient can be removed from isolation with

1. demonstration of virus neutralizing antibodies by RFFIT or equivalent test > 3.0 IU,
   -- AND --
2. Absence of rabies virus genome on 3 serial saliva samples, when samples are rapidly frozen (at bedside) and kept at -80C until analyzed by PCR at a rabies reference laboratory.
   Note: when all PCRs of saliva are negative, but the virus was amplified by PCR from skin or other tissue, then 3 serial negative samples of saliva still are grounds for removal of a seropositive patient from isolation

G.4. Rehabilitation

The natural history of rabies includes almost complete paralysis and sensory loss, as well as significant deconditioning as a result of prolonged intensive care. It is essential that the patient be referred to an excellent rehabilitation service.

All rabies patients so far tested had acquired deficiencies in tetrahydrobiopterin (BH4), with evidence for deficient dopamine and serotonin metabolism. Survivors show pronounced movement disorders that also compromise speech, oral nutrition, sleep, and other activities of daily living.

MODIFIED PROTOCOL (version 3.1)

1. Patients surviving rabies must be tested for BH4 deficiency soon after survival is expected.
2. Patients with documented BH4 and/or dopamine deficiency should be supplemented with enteral formulations of BH4 (10-20 mg/kg/day) for 3-6 months to fully enable rehabilitation.
3. Patients supplemented with BH4 should be tested for therapeutic response by lumbar puncture.
4. A third lumbar puncture should be done several weeks after tapering oral supplementation to confirm that further supplementation is not indicated.
5. See Sections A.5 and E for further details.

H. Determination of futility of care

H.1. Brain death

Rationale

Absent clear brain death, manifest by severe dysautonomia and refractory cerebral edema, it is difficult to use conventional criteria (exam, EEG) in rabies to declare futility of further medical care. Rabies pathogenesis includes encephalopathy with peripheral neuropathy that may falsely mimic brainstem death (see A.4. Denervation and misinterpretation of brainstem death). Interval neurological examinations are therefore of

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unclear utility, and may be deleterious if they precipitate fatal dysautonomia during the first 10 days of hospitalization.

Brain death typically leads to cardiac arrest or refractory hypotension and multi-organ system dysfunction within hours.(35) Brain death in rabies behaves similarly. After significant adverse events-- often related to rabies type 2 vasospasm-- patients progress to refractory cerebral edema over several days.

- A decline in EEG associated with acute spasm of the cerebral arteries is, in our experience, reversible by BH4 supplementation and likely so using "Triple H therapy" or nicardipine.
- Deficiencies of neurotransmitters is reversible by BH4 supplementation, but we do not know if declines in EEG are associated with deficiencies of neurotransmitters and are reversible.(4;5)
- Brain blood flow by HMPAO scan in rabies is often normal. Normal cerebral blood flow has been encountered in human rabies with a significant loss of cortical neurons. (99)
- **The only clear way of withdrawing care, in the presence of cerebral blood flow, is to perform a brain biopsy with the intent of establishing the presence of a full complement of cortical neurons.**

MODIFIED PROTOCOL (version 2.1)

1. Heavy sedation is induced at time of diagnosis of rabies and maintained for 5-10 days, then tapered when (a) there is evidence for denervation of the heart, hence lower risk for dysautonomia (See B. Neuroprotection and therapeutic coma), or (b) there is evidence of neutralizing antibody to rabies in the CSF.
    - Aggressive sedation-anesthesia during the first week of rabies is to be maintained in preference to intermittently reducing sedation in order to perform interval neurological examinations.
    - The presence of rapidly increasing CSF neutralizing antibody (>1 and < 10 IU) should be an indication to taper sedation. Ketamine can be weaned by *slowly* halving over 24-hour intervals until a dose of 0.5 mg/kg/h is reached, with a 10% taper of the benzodiazepine every 1-2 days.
2. It is anticipated that the patient might show complete motor or sensory neuropathy at 10-14 days of illness as sedation-anesthesia is tapered. The EEG should be of near-normal voltage and pupillary responses should recover after the tapering of sedation. Paresis and sensory denervation resolve piecemeal over the next 2 weeks, possibly with emergence of movement disorders.

CAUTIONS

3. Neurological examination during the first 3 weeks of intensive care may be falsely consistent with brainstem death (or locked-in syndrome) and should not be considered an indication for withdrawal of medical care.
4. Loss of EEG voltage or flattening of the EEG is consistent with acute cerebral artery vasospasm or cerebral edema. It should NOT be considered an indication for withdrawal of medical care without proving the absence of cerebral blood flow.
5. Atypical or low-flow (vs. classical no-flow) HMPAO brain scans should not be considered diagnostic of brain death in rabies except in association with grossly abnormal CT or MRI findings, or brain biopsy.

H.2 Alternate definition of futility

We have encountered several patients who show a stereotypical pattern in association of type 2 vasospasm that progresses to neuronal loss or refractory cerebral edema. The presence of the following criteria may be interpreted, *based on a very small number of cases*, as criteria for medical futility:

1. Abrupt decline in EEG or BIS monitor amplitude after 10 days of hospitalization

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- with exclusion of an acute explanatory cause, such as acute generalized (type 1) spasm of the cerebral arteries, or cerebral edema following brief cardiac arrest, brief asphyxia or surgery

2. PLUS diabetes insipidus
3. PLUS CSF protein > 250 mg/dL
4. PLUS CSF lactate > 4 mM, when available.

Given that this is based upon a small number of cases, we strongly encourage that biopsy of the cerebral cortex be considered in such cases to confirm irreversible loss of neurons, hence no hope for meaningful recovery.

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Appendix I: Recent general review of ketamine; older detailed review

Reference List

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Authoritative review that points out the evolution away from early “truisms” regarding ketamine adverse effects.

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Very detailed early review, including interesting clinical studies on the chiral enantiomers. Its anticonvulsive effects and low sedation profile were particularly welcome in preeclampsia.

Annotated references on safety of ketamine for use with increased ICP in humans

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First of 3 studies by same group on match of ketamine + propofol. Uncontrolled, n=8. No differential effect of doses (1.5, 3, 5mg/kg) – all effective. Alters the EEG.

(3) Belopavlovic M, Buchthal A. Modification of ketamine-induced intracranial hypertension in neurosurgical patients by pretreatment with midazolam. Acta Anaesthesiol Scand 1982; 26(5):458-462.

Early study of neurosurgical patients, after pretreatment with midazolam or diazepam. Uncontrolled, n=15. Increases in all 10 midazolam patients, with dose-equivalents 3-fold less than those used for diazepam, little increase in 5 diazepam patients. Recommend against use with reduced intracranial compliance.

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Third of 3 studies by same group. Controlled trial, n=30, of sufentanil-midazolam vs ketamine-midazolam using therapeutic monitoring of plasma concentrations. No ICP problems.

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Second of 3 studies by same group. Controlled trial, n=25, of continuous infusions of ketamine-midazolam vs sufentanil-midazolam. Comparable ICP and CPP. Doses 4.9 mg/kg/h ketamine + 0.98 mg/kg/h midaz (ours 2.0 and 1-3.5, respectively).

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Controlled trial, n=35, of ketamine-midazolam vs fentanyl-midazolam. Equal withdrawals to barbiturate coma for increased ICP. Two cardiovascular arrests in ketamine arm, but higher CPP and better enteral feeding in same arm. Comparable outcomes.

Appendix II: Anticipation of complications in rabies

Table. Complications experienced regularly with rabies. Case reports and reviews of human rabies from 1980 to 10/19/2004 identified through Pubmed were reviewed. More recent experience with the Milwaukee Protocol is added.

Medical complication	Hospital day of onset	Proposed therapy Alternate therapies
Acute phase	**Days 1-6**	
Dehydration from hydrophobia, spasms	1	**Fluid correction using isotonic saline or equivalent** *Caution: Vasopressor use may cause unopposed vasoconstriction of cerebral arteries in setting of BH4 deficiency.* Use of vasopressor mandates daily transcranial doppler (TCD) monitoring. Supplementation with 80 mg BH4 (pediatric: 2 mg/kg/day) also recommended when vasopressors are used.
Syndrome of inappropriate antidiuretic hormone (SIADH). This is often subtle in rabies when assessed by urinary output, and is frequently complicated by cerebral edema. (Classically, this converts to Diabetes Insipidus, below)	1-5	**1. Restriction of free water** **2. Target serum sodium > 140 mEq/L** for first 2 weeks of hospitalization. Monitoring for increased intracranial pressure. *Note: given the dysautonomia of rabies and potential loss of cerebral autoregulation with BH4 deficiency, monitoring for ICP may be best done either directly by intraventricular drain or indirectly by assessment of resistive index by daily TCD.*
Fever Fever is beneficial in accelerating recovery from most viral infections. We tolerated fevers up to 39.5C in our survivor without intervening with medication. (See poikilothermia, below).	1-10	**1. Tolerate fevers up to 39.5C.** **2. Modifying ambient room temperature** for severe hypothermia (<36.0C) or hyperthermia (>39.5C). It is not clear that antipyretics (acetaminophen, ibuprofen,

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Others argue that fever increases cerebral metabolic demands, that should be minimized in a diseased brain.		ketorolac) work in rabies. However, one patient showed baseline hypothermia (<36C) when acetaminophen was administered for pain.
Autonomic instability: tachycardia or supraventricular tachycardia; hypertension. Caution: *This progressed within seconds to bradycardia or asystole in 3 of first 10 patients.* Caution: This may lead to some myocardial stunning, even elevation in CK MB fraction and troponin I. *Note: We have theoretical concerns that early panic, agitation, hypertension and tachyarrhythmia partly reflect catecholamine storm. This will cause the peculiar combination of* ***hypertension (seen in 70%) + left ventricular failure, often with pulmonary edema****(100;101). It has not been directly investigated in rabies. Consider spot blood or urinary catecholamines to diagnose this condition. Treatment of catecholamine storm requires a vasodilator± milrinone, consideration of sympathetic blockers (alpha- and/or beta-blockade).*	1-4	1. **Increased sedation-anesthesia.** *This works very well.* 2. **Maintain hemoglobin > 10 mg%**, appropriate volume loading, and mechanical ventilation targeting arterial normoxia and mild hypercapnia 3. **Central venous pressure monitoring** 4. Cardiac echo to assess myocardial function. (Contractility can be depressed to 45% EF initially, but improves over next several days.) 5. Serum troponin I
Autonomic instability: Bradycardia and asystole; complete heart block. Bradycardia and heart block occur out as far as 10 days of hospitalization, roughly contemporary with advent of the humoral immune response. *Caution: Bradycardia can be associated with sudden decreases in sedation (e.g. abrupt drop of 30% of existing ketamine dose).* *Note: We theorize that sedation can be tapered once the minute-by-minute variation in heart rate and blood pressure are lost.*	4	1. **Increased sedation-anesthesia** *This works very well.* 2. **Maintain hemoglobin > 10 mg%,** appropriate volume loading, and mechanical ventilation targeting arterial normoxia and mild hypercapnia 3. Electrical pacing. *Note: Bradycardia and asystole are not fully rescued by pacing but can respond to increased sedation.* 4. Atropine *during the first week of hospitalization.* *Caution: atropine works paradoxically during the neuropathy phase, dropping heart rate (below)* *Caution: Isoproterenol may be relatively contraindicated*

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		given its effects on intracerebral pressure. Its use should be limited in dose and duration.
Fluctuations in blood pressure (associated with spasms; less severe in week 2) Note: The organ with the 3rd highest concentration of BH4 in the body –after liver and brain—is the adrenal. We hypothesize that there may be a loss of catecholamines from the adrenal medulla. We have observed that blood pressure stabilizes after BH4 supplementation.	1	1. **Increased sedation-anesthesia** **2. Volume management** **3. BH4 supplementation** at 80 mg/day (pediatric 2 mg/kg/day).
Hypersalivation (up to 1.5 L/day) We have also noted copious gastric secretion over several days in week 2-3 (below). Saliva may be very viscid "ropey" – similar to pertussis secretions – and require bronchoalveolar lavage to open up occluded lung segments. *Note: the salivary gland is the only organ where both sympathetic and parasympathetic stimulation lead to sialorrhea. We suspect that the stimulus is sympathetic and that anticholinergics may not work and may be contraindicated with risk of tachyarrhythmia.*	1-6	**Intubation or tracheotomy** *Caution: Avoid elective tracheotomy during period of risk for vasospasm (hospital day 6-14; below)*
Respiratory failure (apnea or non-specific pulmonary dysfunction)		**Intubation or tracheotomy**
Ileus Vomiting is common in rabies, along with hypersalivation, but distinct from ileus, which usually lasts only 5 days at time of maximal denervation.	1-8	**1. Nasojejunal tube for feeds** and to instill oral medications 2. Parenteral nutrition
Urinary retention *This is a classic finding discriminating rabies from Guillain-Barre syndrome.* *Caution: this may cause spinal reflex dysautonomia.* *Note:* clogged urinary catheters have been common in our experience.	4	1. **Bladder catheterization** 2. Consider interval bladder irrigation or surveillance by bladder ultrasound
Inspiratory spasms	1-4	1. **Increased sedation – anesthesia**

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Apneustic breathing and spasms have been noted despite sedation that suppressed the EEG. We presume that this reflects continued dysautonomia at the brainstem level. *Spasms may be tolerated during medical care when infrequent, although this in uncomfortable for the family and staff. Usually spasms lead to triggering of the ventilator. Intermittent paralysis is an option.*		*Note: spasms may respond to more lipid-soluble benzodiazepines (diazepam, alprazolam).* 2. **Consider intubation to avoid aspiration**. 3. **Avoidance of tracheal stimulation** (lidocaine applied to trachea) 4. Paralysis when needed. *Note: complete paralysis will follow (anyway) in natural rabies by 10 days (see below).*
Focal seizures It is not clear from the literature whether there is an EEG correlate ***Note: we have only seen seizures in setting of complications, such as cerebrovascular spasm (below).*** *Caution: Barbiturates inhibit lymphocyte proliferation at physiological concentrations (20-30 mg/dL).* We need immune response to clear rabies. Anecdotally, immune response in several patients was delayed following heavy use of barbiturates. Loading doses correlated with transient delays in titer evolution, more than serum concentrations, in another patient. *Caution: 2 patients receiving topiramate in context of ketamine-benzodiazepine-induced coma in rabies showed complete flattening of EEG within hours.* Obviously confounded and anecdotal, but would avoid when possible.	1-4, 15	1. **Confirm that there is sufficient intracranial perfusion by emergent TCD.** **2. Confirm seizures by EEG when possible.** **3. Anticonvulsants**. Note that benzodiazepines and ketamine are effective anticonvulsants that do not suppress the EEG. Gode used diphenylhydantoin and vitamin C, with 2 reported survivors; consider fosphenytoin for better bioavailability for critical care.

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Progressive phase	**Days 7-14**	
Diabetes insipidus (5-15 L/day) DI is intermittent, cyclical --often with 36 hour periodicity—and usually mild in rabies *Caution: We have only encountered severe DI in rabies after severe complications, such as stroke.* *Caution: In a busy ICU, it is often impossible to keep up with changes in DI unless a sliding scale is used. For same reason, arginine vasopressin is recommended over DDAVP.* *Caution: Patients can be exquisitely sensitive to AVP.*	4-14	1. **Urinary catheter** **2. Exclude salt wasting (below)** **3 Emergent TCD and head CT** 4. **Sliding scale mL/mL replacement of excess losses** (say >2-3 cc/kg/h) with 1 milliUnit arginine vasopressin/ 500 mL final volume of 1:1 mix of 0.45N saline and D5W (=D2.5 0.2NS). Losses should be assessed and replaced every 3-4 hours. *Caution: AVP is also a vasopressor. Vasopressor use may cause unopposed vasoconstriction of cerebral arteries in setting of BH4 deficiency.* Use of vasopressor mandates daily transcranial doppler monitoring. Supplementation with 80 mg BH4 (pediatric: 2 mg/kg/day) also recommended when vasopressors are used.
Generalized flaccid paralysis. EMG can reflect either axonal injury or demyelination.	onset 4-8 complete by day 10-14	1. **ventilation**; 2. **frequent repositioning to avoid pressure sores** 3. **heparin prophylaxis** *Note: Low molecular weight heparin is not easily reversible if a neurosurgical procedure is required emergently.* 4. **physical therapy** to prevent contractures
Adrenal insufficiency Lack of response to cortisol stimulation test in a single patient, after cardiac arrest	5	
Increased CVP Mechanism unknown. Good cardiac function by echocardiography, which is relatively insensitive for this problem	5-21	Consider supplementation with 80 mg/day BH4 (pediatric: 2 mg/kg/day)

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Generalized cerebral artery (and ICA) spasm, rabies type 1 Frequency uncertain. This is predicted to reduce cerebral blood flow to 50% of normal (limit of pressure autoregulation). Severe spasm by TCD has been associated with transient loss of EEG amplitude and cerebral edema the following day.	6-10	**1. Prophylax with nimodipine.** • Enteric dose: 60 mg PO Q4h x 21 days. [This is about 1.5 mg/kg/dose in children.] • Can be given sublingually to titrate. • IV formulation (adults and children): start 7.5-10 mcg/kg/h initially, increasing over a few hours to 30 mcg/kg/h (max 45 mcg/kg/h). This gave hypotension in 24% 2. Confirm doppler studies with CT angiography. 3. When spasm present, consider • ventriculostomy to monitor ICP • IV nicardipine 75 mcg/min (pediatric dose: 0.5 mcg/kg/min) to treat spasm • OR: "triple H therapy".
Conjunctival suffusion This has been seen regularly and is unexplained. There are migraine variants with this finding, raising the question of whether it is a marker of brain vascular dysfunction.(102)	6-10	
Increased intracranial pressure (ICP). This likely follows hypoxia-ischemia, rather than being caused by rabies virus infection per se. (See also Subacute cerebral edema, below.) Near infrared spectroscopic monitoring (NIRS) has clearly missed early detection of cerebral edema in rabies managed by Milwaukee Protocol, perhaps because of reduced brain metabolism by general anesthesia or different sensitivities of anterior and middle cerebral artery territories.	6-11	**Intraventricular drain** will permit (a) monitoring of ICP, (b) therapeutic removal of cerebrospinal fluid Note: Biopsy of brain cortex at time of drain placement should be strongly considered if rabies diagnosis is still in doubt OR if there are concerns about medical futility that can be relieved by normal histology on biopsy.
Loss of somatotrophic activity (growth hormone) reported in various species with rabies, early in disease	NOT documented in humans	

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False mimicry of brainstem death (severe encephalopathy + complete radiculopathy) EEG, MRI and cerebral blood flow are generally preserved	7-12, 20	**Continue support**
Advent of rabies specific neutralizing antibody in serum. Advent of *CSF neutralizing antibody* follows by 1-2 days. Evolution of CSF neutralizing antibody appears to be more delayed relative to serum antibodies in dog-associated rabies. ***Note: In setting of neutralizing antibody to rabies virus > 3 IU/ml, three (3) negative saliva samples are sufficient to remove a patient from isolation.*** Samples must be well collected, on different days, and tested by a rabies reference laboratory.	7-12	1. **Daily or every other day serology** to monitor advent and progression of immune response. 2. **Daily or every other day collection of saliva** (or buccal swabs) in 1-3 ml of viral media (or Trizol) to document clearance of virus.
Subacute cerebral edema *In bat-associated rabies*, increases in CSF neutralizing antibody titers > 10 IU have been associated with onset of a subacute form of cerebral edema that can persist for 3 weeks. *Caution: subacute cerebral edema appears to be frequently complicated by salt wasting (below).*	10-33	1. Consider baseline CT or MRI 2-3 days after detection of neutralizing antibody in serum. 2. Maintaining serum sodium > 140 mEq/L for 1-2 weeks using dietary sodium supplementation or hypertonic saline, with fixed free water administration. (See A.12 Nutrition) 3. Antibody rise can be fully stopped using 10-30 mg/kg pulse of methylprednisolone or 1 mg/kg pulse of dexamethasone x 3 days. A 24h pulse may be preferable.
Salt wasting (SW) Cerebral salt wasting is a controversial diagnosis. SW in rabies may be nephrogenic. Salt wasting has been encountered in 3 successive (bat) rabies patients since elimination of ribavirin from the protocol. Two patients showed subacute cerebral edema (see above) and management was complicated by SW. One of these was noted retrospectively to be receiving	10-33	1. **Avoid diuretic use** 2. **Monitor serum and urine sodium and uric acid** at intervals from day 7-14 to accurately diagnose the disorder. 3. For Na < 140, consider enteral supplementation with salt tablets: 1 gm salt/5 cc water by nasogastric or nasojejunal tubes 2-3 times daily, or by admixture into isotonic enteral

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diuretics. That patient did not respond to a challenge with vasopressin, suggesting renal salt wasting. A third patient (vaccine failure) showed moderate edema of basal ganglia and centrum semiovale white matter in association with SW. *Caution: Sodium replacement to increase serum sodium concentrations reached 20-30 mEq/kg/day in these cases.* Note: rabies virus antigen was detected in the renal tubules of patients during an outbreak associated with solid organ transplantation.(103)		nutrition during second to fourth weeks of hospitalization. 4. Higher doses of enteral supplementation with sodium can be minimized by use of mineralocorticoid supplementation with fludrocortisone 0.15 mg daily (children 0.1 mg daily).(104;105) Hydrocortisone also has mineralocorticoid activity but may suppress immune response.
Hypothyroidism: Low TSH, low T3, low T4, normal T3RU on T4 supplementation was seen in a single patient with gradual declines in pulse, blood pressure and temperature in second week, after cardiac arrest and with normal TSH and T4 in first week Note: one of the earliest signs of hypothyroidism is increased peripheral vascular resistance (associated with type 2 cerebral artery vasospasm).(106;107)	7-21	
Bradycardia and asystole; need for electrical pacing; complete heart block We have seen this in temporal association with advent of rabies antibody in serum. Recovery from heart block has been reported in longer-surviving patients in reports preceding use of the Milwaukee Protocol. *Caution: There is usually no atropine effect at this stage, presumably indicative of a lack of vagal innervation of the heart. Response to atropine may be paradoxical (bradycardia).*	7-27	1. **Maintain hemoglobin > 10 mg%**, appropriate volume loading, and mechanical ventilation targeting arterial normoxia and mild hypercapnia **Check ventilation** 2. **Trial of increased sedation** 3. **Transvenous pacemaker** Note: we have had intravenous pacemakers float away during lumbar puncture or patient repositioning. We wonder whether placement of epicardial wires may be a better option, when possible. *Caution: Use of isoproterenol during heart block can lead to over-perfusion of the cerebral vasculature, with incipient cerebral edema. Use should be minimized.* 4. **Check for serum antibody to rabies**.

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Denervation (by nerve conduction studies) Sensory & Motor	11-12	
Poikilothermia Most increases in temperature after the first week were related to high ambient temperature, or use of support hose and inflatable stockings, EEG electrode covering, or other bedding that minimized radiant heat loss by patient. *Caution: We have regularly seen no effect of antipyretics in this setting, including acetaminophen, ibuprofen and ketorolac.* However, one patient showed baseline hypothermia (<36C) when acetaminophen was administered for pain.	11-12	1. **Poikilothermia requires manipulation of room temperature** 2. **Evaluate temperature of ventilator circuit.** 3. Frequently cycle use of support hose/stockings to improve radiation of heat
Coma Progressive flattening of EEG is part of the natural history of rabies, but its mechanism is not known. *Caution: Acute flattening by EEG have been correlated mostly with spasm of intracranial arteries by TCD. (See vasospasm, below) Some medications (topiramate, propofol) have also been associated.* Theoretically, the EEG can also go flat through loss of catecholamine neurotransmitters after loss of BH4. Loss of HVA (dopamine metabolite) in CSF correlated with loss of EEG activity in one patient.	11-12	1. **Obtain TCD emergently** to confirm adequate perfusion. 2. If insufficient perfusion (Vmean < 30 cm/s) with high resistance index or pulsatility index, obtain **emergent CT ± CT angiogram** to exclude cerebral edema and confirm vasospasm. A. If low velocity/high resistance without edema by CT: 1. Consider IV **nicardipine** 75 mcg/min (pediatric dose: 0.5 mcg/kg/min). Watch mean arterial pressure. 2. OR: Consider "triple H therapy" 3. Consider **ventriculostomy** to monitor ICP Strongly consider brain biopsy if intracranial pressure abnormally low. 4. **Lumbar puncture** for cell count, protein, glucose, lactate & pyruvate, rabies antibody titers, pterins (biopterin, neopterin), HVA and 5-HIAA. B. If there is cerebral edema by CT, then treat this through standard interventions of modest hyperventilation (PaCO2 30-35 mm Hg) and hypertonic saline, elevated head of bed, etc. *Hyperventilation will work even if BH4/NOS pathway is compromised, in humans.(108)* *Caution: hyperventilation will constrict vessels much more*

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		dramatically in setting of an absence of vasodilator tone from nNOS. Would strongly recommend that blood flow be monitored by TCD during hyperventilation. 3. If EEG is flat and there is sufficient perfusion by TCD and no cerebral edema, then 1. **Lumbar puncture** for cell count, protein, glucose, lactate, rabies antibody titers, pterins (biopterin, neopterin), HVA and 5-HIAA. 2. **Consider brain biopsy** to confirm normal neuron densities.
Generalized cerebral artery (and ICA) spasm, type 2 Uncertain prevalence. *We have only seen this when vasospasm, type 1, was encountered previously. It is ominous.*	12-17	1. Consider **nimodipine prophylaxis** x 21 days. 2. Screen for CSF lactate before this date. 3. Screen for hypothyroidism before this date. 4. Consider IV **nicardipine** 75 mcg/min (pediatric dose: 0.5 mcg/kg/min). Watch mean arterial pressure. 5. OR: Consider "triple H therapy" 6. Consider **ventriculostomy** to monitor ICP Strongly consider brain biopsy if intracranial pressure abnormally low. 7. **Check for rabies-specific immune response (serum)** 8. **Lumbar puncture** for cell count, protein, glucose, lactate & pyruvate, rabies antibody titers, pterins (biopterin, neopterin), HVA and 5-HIAA.
Skin flushing or urticarial rash We do not know the mechanism of this finding, but it appears to be associated with **onset of the immune response**, and has been associated with diminution in rabies epifluorescence on skin biopsy and viral load in saliva by RT-PCR.	12-25	**1. Check for rabies-specific immune response (serum)** **2. Consider skin biopsy for rabies DFA ± PCR in 2-3 days**

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Note: the fifth BH4-dependent enzyme pathway –alkylglycerol monooxygenase -- involves the degradation pathway of the metabolic pool for platelet activating pathway (PAF). PAF and histamine are major mediators of urticaria, and may contribute to local tissue edema as well as hypotension. It may be worth looking for PAF, histamine or serum tryptase in these patients, given that there are therapeutic interventions such as antihistamines that have not been considered. Alprazolam (a benzodiazepine) and lupatadine (a leukotriene inhibitor) are PAF-receptor blockers licensed in certain countries.		

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Terminal phase	**Days 14-19**	
Markedly **reduced left ventricular ejection fraction; myocarditis**. *Note: the evidence for rabies-specific myocarditis is poor. Histochemically, most rabies is restricted to the conduction system.* *Caution: decreased LV function and hypotension are found after brain herniation.(35)*	6-21	1. **Brain CT, MRI** or HMPAO scan to exclude cerebral edema with herniation.
Hypotension We have encountered true hypothyroidism – not sick euthyroid -- in the second to third week of illness <u>in one patient</u>, after cardiac arrest in week 1. *We believe that hypotension is related in part to failure of the adrenal medulla from rabies-associated biopterin deficiency.* The fifth BH4-dependent enzyme pathway –alkylglycerol monooxygenase -- involves the degradation pathway of the metabolic pool for platelet activating pathway (PAF). PAF is a major mediator of anaphylaxis, third-spacing and hypotension. It may be worth looking for PAF, histamine or serum tryptase in these patients, given that there are therapeutic interventions for anaphylaxis, such as antihistamines, that have not been considered. Alprazolam (a benzodiazepine) and lupatadine (a leukotriene inhibitor) are PAF-receptor blockers licensed in certain countries. *Caution :decreased LV function and hypotension and endocrinopathy are found after brain herniation.(35)*	7-24	1. **Cardiac echo** 2. **Vasopressor support** *Caution: vasopressor use may cause unopposed vasoconstriction of cerebral arteries in setting of BH4 deficiency.* Use of vasopressor mandates daily transcranial doppler (TCD) monitoring. 3. Consider **supplementation with 80 mg BH4** (pediatric: 2 mg/kg/day). 4. Many clinical aspects of rabies overlap with the clinical syndrome of brain death. Consider the endocrine cocktail pioneered in care of organ donors: thyroid supplementation, methylprednisolone, ± arginine vasopressin.(35)
Absence of cortical activity by EEG	complete by day 14-24	1. **Obtain TCD emergently** to confirm adequate perfusion. 2. If insufficient perfusion (Vmean < 30 cm/s)

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		with high resistance index or pulsatility index, obtain **emergent CT ± CT angiogram** to exclude cerebral edema and confirm vasospasm. A. If low velocity/high resistance without edema by CT: 1. Consider IV **nicardipine** 75 mcg/min (pediatric dose: 0.5 mcg/kg/min). Watch mean arterial pressure. 2. OR: Consider "triple H therapy" 3. Consider **ventriculostomy** to monitor ICP Strongly consider brain biopsy if intracranial pressure abnormally low. 4. **Lumbar puncture** for cell count, protein, glucose, lactate & pyruvate, rabies antibody titers, pterins (biopterin, neopterin), HVA and 5-HIAA. B. If there is cerebral edema by CT, then treat this through standard interventions of modest hyperventilation (PaCO2 30-35 mm Hg) and hypertonic saline, elevated head of bed, etc. *Hyperventilation will work even if BH4/NOS pathway is compromised, in humans.(108)* *Caution: hyperventilation will constrict vessels much more dramatically in setting of an absence of vasodilator tone from nNOS. Would strongly recommend that blood flow be monitored by TCD during hyperventilation.* 3. If EEG is flat and there is sufficient perfusion by TCD and no cerebral edema, then 1. **Lumbar puncture** for cell count, protein, glucose, lactate, rabies antibody titers, pterins (biopterin, neopterin), HVA and 5-HIAA. 2. **Consider brain biopsy** to confirm normal neuron densities.
SVC clots	22	1. **Heparin** 1000 IU BID or 10U/kg/h. *Note: Low molecular weight heparin is not easily*

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		reversible if a neurosurgical procedure is required emergently. 2. Support stockings and/or pressure boots.