Dengue Hemorrhagic Fever: Pathology and Pathogenesis
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The University of Texas Medical Branch
Galveston TX

Dengue Hemorrhagic Fever is the most severe manifestation of human infection by the mosquito-borne flavivirus Dengue. Dengue virus is an enveloped virus, with a single stranded, positive sense RNA genome that encodes three structural genes (E, PrM, C) and seven nonstructural genes. There are four antigenically distinct serotypes of Dengue virus (DEN1-4). Geographic expansion of the range of dengue serotypes and the *Aedes aegypti* vector has been accompanied by dramatically increasing numbers of Dengue fever and DHF cases. DHF is distinguished from classic Dengue Fever (DF) by the presence of vascular leak, manifesting as hemoconcentration, hypoproteinemia, serous effusions, and in the most severe cases, shock. DHF has been classified into four grades based on clinical indicators, with grades III and IV representing Dengue shock syndrome (DSS). DHF occurs most commonly in children and is associated with secondary infection by a heterologous Dengue serotype. DHF is generally associated with higher viremia titers than DF. Thrombocytopenia is a constant feature of dengue infections, but the mechanism of this is not clear. DIC is seen in only a few instances of grades III-IV DHF. Plasma leak coincides with defervescence and clearance of viremia, suggesting immunopathological mechanism of endothelial injury as opposed to direct effects of virus.

The pathology of fatal DHF has been well described in large autopsy series. Hemorrhages of the pleura, epicardium, gastrointestinal mucosa and skin are present, and serous effusions and edema of retroperitoneal soft tissues are prominent. Histopathologic manifestations are dominated by the liver lesion, which consists of variable degrees of hepatocellular necrosis, primarily midzonal. Other features of the associated hepatitis, such as presence of Councilman bodies and Torres bodies are reminiscent of Yellow Fever. Spleens show atrophy of the white pulp, both T and B cell areas, along with increased numbers of reactive lymphocytes in the red pulp, correlating with the presence of atypical lymphocytes in the peripheral blood. Capillaries and arterioles in several organs show endothelial swelling, minimal perivascular inflammation and edema, and rare apoptotic endothelial cells. In general, histopathologic changes do not explain the profound microvascular insufficiency characteristic of this disease.

Dendritic cells and cells of the mononuclear phagocyte system are important early targets of infection. Immature Langerhans cells are permissive for infection and are likely the earliest target after infection by the bite of an infected mosquito. Antibody dependent enhancement of monocyte infection has been demonstrated in primary unfractionated cultures of human peripheral blood leukocytes and splenocytes infected with various DEN isolates. In tissues obtained at autopsy or biopsy, immunohistochemistry demonstrates viral antigen in hepatocytes, Kupffer cells, splenic macrophages, and, focally, in endothelial cells.
DHF is believed to be immunologically driven. The Halstead hypothesis states that secondary infection by a different Dengue strain results in antibody dependent enhancement of mononuclear phagocyte infection. Secondary dengue infections are also associated with generation of cross-reactive T cell responses originating from T memory cells. Severe disease is associated with immunological activation markers, such as sIL-2R, IL-2, and activated immunophenotype of peripheral blood monocytes. The degree of liver injury correlates not with viremia, but with markers of immune activation. Mechanisms of endothelial injury are likely multiple and include direct viral effects and indirect effects of cytokines and other mediators. Overproduction of inflammatory cytokines, such as IFNγ, TNFα, MCP-1, and IL-8 has been documented in serum of DHF patients. Monocyte/macrophages and activated T cells are among the probable sources of these mediators. Antibodies generated against the viral NS1 protein cross-react with microvascular endothelial cells and may initiate endothelial injury. Infected endothelial cells show altered expression of VEGF receptors and matrix metalloproteinases, which participate in the regulation of endothelial permeability. The viral protein NS1 interacts with the complement inhibitory protein clusterin, suggesting alterations in complement regulation. The identification of of cross-reactive anti-E antibodies that bind plasmin peptides suggest possible interference with fibrinolysis/coagulation systems. Any explanation of vascular leak syndrome in DHF must take into account the relatively sparse infection of microvascular endothelial cells and paucity of frank endothelial damage in fatal human cases. Because animal models that recapitulate the natural history and pathology of DHF are not available, significant gaps remain in understanding the kinetics and sites of viral replication and their relationship to plasma leakage syndromes.
Reference List


Dengue hemorrhagic fever is an acute febrile syndrome with vascular leak, caused by the widely distributed, mosquito born flavivirus Dengue. DHF is most commonly seen in children experiencing a secondary infection with a heterologous serotype of dengue virus. Seroepidemiologic and immunologic studies suggest that pre-existing, non-neutralizing antibody enhances infection of target mononuclear phagocytes and increases virus replication.

The pathology of fatal DHF has been well described in human autopsy series, but information on the kinetics and location of viral replication in tissues during natural infection is sparse. Animal models that faithfully recapitulate the all aspects of the natural history and pathology of DHF are not available.

Major pathologic findings are hemorrhages, edema, and midzonal, paucicellular necrosis in the liver. Large reactive lymphocytes are seen in peripheral blood and lymphoid tissues. There are small foci of mild perivascular inflammation, edema, and endothelial swelling in microvasculature of many organs. Important target cells include dendritic cells, mononuclear phagocytes, hepatocytes, and focally, endothelial cells.

Suggested mechanisms of vascular leak include overproduction of pro-inflammatory cytokines by activated T cells and monocyte/macrophages, direct viral effects on regulation of endothelial permeability, alterations in regulation of complement and fibrinolytic systems, and antiviral antibody that cross-reacts with endothelial cells.

Keywords: Dengue virus, hemorrhagic fever, cytokines, endothelium, immunopathology, hepatitis
Importance of microvesiculation in the immunopathology in cerebral malaria

Georges E. R. Grau, *M.D., Ph.D.*

Vascular Immunology Unit
The University of Sydney
Australia
microparticles:

produced by microvesiculation of plasma membranes
Approach to cerebral malaria pathogenesis

cytokines / receptors

platelets

microparticles
Overview / microparticles (MP)

- what are they?
- do they change in CM?
- what happens if they are blocked?
- what do they do to their targets?
TNFα or TNFβ (lymphphotoxin)?

Grau et al., Science 237: 1210, 1987
Rae et al., FASEB J 18: 499, 2004
Effector cells

monocyte

CD8+ T cell

mTNF

mLTα1β2

mLTα2β1

sLTα3

ICAM-1 upregulation

Endothelial cell

TNFR2

Unknown target cell

Effects?

Trends in Immunology
Hunt & Grau, 2003

Togbe et al.
PLoS One 2008
MRI assessment of brain swelling in mice with CM

Intravascular platelet binding in CM: IHC and Ligand-Induced Binding Sites

LIBS: detection of MRI-invisible lesions

von zur Mühlen et al., J Clin Invest (2008)
In vivo arguments in favour of a pathogenic role of platelets in microvascular pathology

- platelets sequester in the organs where lesions will occur
  - cerebral malaria
  - pulmonary fibrosis
  - Shwartzman reaction, LPS shock
  - DTH

- anti-LFA-1 mAb blocks platelet sequestration and pathology

- platelet depletion prevents microvascular damage and mortality

Modelling human cerebral malaria

in vitro

1. Immunostaining (patient brain)

2. Cell isolation

3. Co-cultures

BRAIN endothelial cell

PRBC

WBC

platelets
Platelets bind to TNF-activated endothelium and potentiate its apoptosis

1. Direct killing
2. Indirect killing

Endothelial activation/sensitisation

Wassmer SC et al., J Immunol 176: 1180-4, 2006
Platelets bind to TNF-activated endothelium and potentiate its apoptosis

Wassmer SC et al., J Immunol 176: 1180-4, 2006
2 levels of complexity

cell-cell interactions

+ cell-derived microparticles
Microparticle production: membrane vesiculation

Zwaal et al, Blood 96
Vesiculation: microparticle (MP) production

- TNF
- A23187
- LPS
- ADP

MP

<1 µm

surface Ags from the cell of origin

⇒ proadhesive and procoagulating properties

△ membrane polarity

△ phospholipid asymmetry

cell surface
MP phenotype is the image of its ‘mother cell’

ICAM-1

Brain EC

EMP

non stim.    TNF
Both TNF and LTα enhance MP production by human endothelium

Combes et al., *J. Clin. Invest.* **104**: 93-102, 1999

Wassmer et al., *IAI*, **74**: 645-653, 2006
Overview / microparticles (MP)

• what are they?

• do they change in CM?

• what happens if they are blocked?

• what do they do to their targets?
Dramatic increase of plasma endothelial microparticles in Malawian children with CM

+ EMP correlate with TNF in CM patients (acute phase)

Combes et al, *JAMA* 291: 2542-4, 2004
Are microparticles pathogenic?
Experimental cerebral malaria (murine model)

Infection: *Plasmodium berghei ANKA*

- **Endothelial activation, cytoadherence**
  - Days: 0, 7, 14
- **Anaemia, hyperparasitaemia**
  - Days: 14, 21

- **Death (CM-resistant)**
- **Death (CM-susceptible)**

Days on the timeline range from 0 to 21.
MP levels are higher at the time of CM in susceptible mice

![Graph showing MP levels in Swiss and C57BL/6 mice with and without PbA treatment. The graph indicates significant differences with p-values of 0.001 and 0.01 for Swiss and C57BL/6 mice, respectively.](image-url)
Enzymes controlling phospholipid movements

**Outer leaflet**
- PE
- PS

**Inner leaflet**
- amino-phospholipid translocase

Additional enzymes:
- floppase
- scramblase
Approach to genes

- hypothesis-driven
- non hypothesis-driven
- SERENDIPITY
ATP Binding Cassette Transporter A1

Deleted segment in KO mice
(Hamon et al. Nat Cell Biol. 2: 399, 2000)

Human Pathology (Tangier Disease)
ABCA1 gene deletion prevents CM

ABCA1+/+ (DBA/1)

ABCA1-/- (DBA/1)

C57BL/6

cerebral phase

Survival (%)

Parasitaemia (%)

0 25 50 75 100

0 25 50 75 100 125 150

10 20 30 40 50 60 70 80 90

0 7 14 21

Time after infection by PbA (days)
Upon PbA infection: higher levels of plasma microparticles in ABCA1\(^{+/+}\) mice

Microparticle quantitation in plasma

Cellular origin of microparticles

Combes et al., *Am J Pathol* 166: 295-302, 2005
Down-regulation of brain inflammation in PbA-infected ABCA1-/- mice

ICAM-1

LFA-1

GPIIb-IIIa

-/+  +/+  -/-  -/-

-/+  +/+  -/-  -/-

-/+  +/+  -/-  -/-

P<0.001

P<0.0001
Microparticles from PbA-infected CM-susceptible mice are more procoagulant than those from PbA-infected ABCA1-KO mice.
Microparticles from PbA-infected CM-susceptible mice are: more inflammatory than those from non-infected CM-S mice.
Overview / microparticles (MP)

- what are they?
- do they change in CM?
- what happens if they are blocked?
- what do they do to their targets?
Can one block MP production?

Yes, by gene deletion: ABCA1 knock-out mice

but how about pharmacologically?
Blocking EC activation?

Stabilising membrane PS?

Stabilising membranes?
Stabilising membrane phospholipids

• Rationale: cell activation = modification of membrane lipids

• Maintaining membrane asymmetry may stabilise lipids

• Code: THP-22
THP-22 treatment of brain endothelial cells

- THP-22 (0 – 5 mM) for 12h
- TNF / LT (100 ng/ml) at 12h
- THP-22 (0 – 5 mM) for 12h
- MP quantification in culture supernatant
THP-22 treatment abolishes human brain endothelial vesiculation *in vitro*
THP-22 treatment prevents CM development

Penet et al., Proc Natl Acad Sci USA 105:1321-6, 2008
<table>
<thead>
<tr>
<th>Question</th>
<th>In Vitro</th>
<th>In Vivo</th>
<th>Protect Against CM?</th>
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<tr>
<td>Can we modulate MP production?</td>
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<tr>
<td>Stabilising membranes?</td>
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<tr>
<td>Blocking EC activation?</td>
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<table>
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<th>In Vitro</th>
<th>In Vivo</th>
<th>保護 Against CM?</th>
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<td>LMP-420*</td>
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<td>nd</td>
<td>nd</td>
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<tr>
<td>THP-22#</td>
<td>+</td>
<td>+</td>
<td>yes</td>
</tr>
<tr>
<td>CTC, DiA</td>
<td>+</td>
<td>nd</td>
<td>nd</td>
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</tbody>
</table>

(#) Penet et al., *Proc Natl Acad Sci USA* 105:1321-6, 2008
Overview / microparticles (MP)

• what are they?

• do they change in CM?

• what happens if they are blocked?

• what do they do to their targets?
What are the downstream effects of MP?
In vitro modelling of human CM

- Cytoadherence
- Morphological features
- Phenotypic changes
PMP bind to and activate HBEC

HBEC

Ø PMP  PMP

DAPI

PKH67

ICAM-1

Ø PMP  PMP  TNF

IgG1

VCAM-1
How?

PMP adhere to and penetrate in brain EC

Brain EC

Adherent PMP

consequences?

Internalised PMP

mechanisms?
MP-target interactions: 3 patterns

“cytosolic”  “surface”  “compartmentalised”: micropinocytosis?
Some PMP are internalised in lysosomes

PMP
PKH67 green

Lysosomes
Lyso-tracker red

90 min incubation
37°C
Platelet MP also bind to PRBC and transfer platelet antigens to their surface.
PMP dramatically increase RBC binding to EC

- **First Condition:**
  - **Ø MPP**
  - **PMP on PRBC**
  - **PMP on EC**

- **Second Condition:**
  - **∅ MPP**
  - **PMP on PRBC**
  - **PMP on EC**

**Graph:**
- **Bound RBC / field**
- **TNF**
  - **-**
  - **+**

**Significance:**
- **p = 0.0002**
- **p = 0.0076**

**Legend:**
- **nRBC**
- **PRBC**

**Notes:**
- HBEC-5i
Membrane fusion / Ag transfer?

MP

Ag (R)

+ ?

cell

morphology?
functions?
signalling?
Differential labelling of membrane versus cytosolic elements for the co-cultures

**HBEC (D3 line)**

- **PRBC-PKH26**
  - **Calcein AM**

- **O/N**
- **1 h 30**
- **0 / 1 h / 2 h**

- **TNF**

- **unbound cell removal**
- **washing**

- **INCUBATION**

- **microscopy**
HBEC: D3 cell line + IRBC: 3Ci

30 min co-culture

40 min co-culture

90 min co-culture (before washing)

Adhesion

Beginning of transfer
1 h 30 min co-culture (after washing)

Diffusion of membrane compounds

PKH26

calcein

HBEC: D3 + IRBC: 3Ci
4 h co-culture

Diffusion of both membrane and cytosolic compounds
Could this transferred membrane material include *P. falciparum* antigens?

**PRBC-PKH26**

**HIS + anti human-IgG**

**HBEC**

1 h 30

0 / 1 h / 2 h

confocal microscopy

**AutoMACS® - purified PRBC**

[HIS : pool of 20 hyper-immune sera from African adults with malaria]
Intracellular localisation

Surface localisation
Roles of microparticles during CM

Antigen transfer

PLT

PRBC

TNF

PMP

Adhesion molecules

Adhesion + EC DAMAGE?

Roles of microparticles during CM

Antigen transfer

PLT

PRBC

TNF

PMP

Adhesion molecules

Adhesion + EC DAMAGE?
MP as players of pathogenesis

- Plasma TNF is high in CM

- TNF enhances endothelial MP release \textit{in vitro}
  \textit{J Clin Invest} 1999

- High plasma MP found in patients with CM
  \textit{JAMA} 2004

- ABC A1 gene knock-out mice
  - Do not show a rise in MP
  - Are fully protected against the cerebral pathology
    \textit{Am J Pathol} 2005

- Treatment with PTTH reduces MP \textit{and} prevents CM.
  \textit{Proc Natl Acad Sci USA} 105:1321-6, 2008
MP in cerebral malaria: CONCLUSIONS

• Are dramatically elevated at the time of CM

• Are pro-inflammatory and procoagulant

• Their blockade prevents lesions (*in vitro*: H; *in vivo*: M)

• Can enhance binding of RBC to brain EC

• Can transfer antigens to EC and alter EC functions
Summary

cytokines / receptors
- TNF-\(\beta\), not TNF-\(\alpha\), is required
- TNFR2 and LT\(\beta\)R are required

platelets
- accumulate at the site of lesions, before these become MRI-detectable
- participate in endothelial alterations

microparticles
- downstream and upstream events
activated cell → microparticles → target cell

- upstream
  - kinetics?
  - cytoskeleton?
  - rafts?

- downstream
  - fusion?
  - internalisation
  - ag transfers
  - adhesiveness
  - procoag.
  - Δ permeability
  - novel signalling?
  - trogocytosis?

pathology?
Bacterial Sepsis

United States and Canadian Academy of Pathology
Binford-Dammin Society of Infectious Disease Pathologists
March, 2009

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Outline for the Sepsis Talk

SIRS – Sepsis - Cytokines

Real sepsis – not endotoxemia

Legitimate animal model of sepsis

6@6, Six at Six

Early deaths, late deaths SIRS→CARS

Multiplexing for more data

Using the changes to alter therapy
SIRS - Systemic Inflammatory Response Syndrome

2 or more of the following are needed

*Temperature*  \( \geq 38^\circ \) or \( < 36 ^\circ C \)

*Heart rate*  \( > 90 \) beats/minute

*Respiratory rate*  \( > 20 \) or \( \text{PaCO}_2 < 32 \text{mm Hg} \)

*WBC*  \( > 12 K \) or \( < 4 K/\text{mm}^3 \), or \( > 10\% \) bands

*Bone Chest 1992;101:1644*
SIRS - Definition Too Broad?

- Sweating - hyperthermia
- Heavy breathing - tachypnea
- Heart racing - tachycardia

The definitions are too broad and encompass a wide range of different activities, not all of which are pathologic.
The Problem of Sepsis

- Reviewed discharges of 750,000,000 patients
- Between 1979 and 2000 the incidence of sepsis increased 8.7% per year
- Gram positive more frequent than Gram negative
- Mortality is greatest among black men

Martin, NEJM 2003:348:1546
Sepsis – The Movie

1999 Movie with
George Clooney
Mark Wahlberg
Ice Cube
Cytokines and Sepsis

• Explosive release of cytokines is responsible for the organ injury and mortality in sepsis
The 4 Pillars of Support
Cytokines Cause Sepsis

- LPS releases cytokines
- Cytokine infusion = sepsis
- Sepsis have high cytokines
- Block cytokines better survival
Cytokine Inhibition for Rx of Sepsis

- In animal models
  - Blocking TNF improves survival
  - Blocking IL-1 improves survival

Proposed patient studies

- Block TNF or IL-1
- Save lives
- So Simple
Cytokine inhibitors for Sepsis

• Based on multiple preclinical trials using animal models of sepsis, large scale clinical trials were initiated
• All trials enrolled patients meeting the SIRS criteria
• All patients received routine clinical care for sepsis (antibiotics, fluids, organ support)
Treatment of Human Sepsis
Monoclonal antibody to TNF

994 total patients
No improvement in survival

Abraham JAMA 1995;273:934
Treatment of Human Sepsis
TNF soluble receptors TNF:Fc

Mortality at 28 days

Placebo .15 mg/kg .45 mg/kg 1.5 mg/kg

141 total patients
Increase in mortality with therapy

Fisher NEJM 1996;334:1697
Treatment of Human Sepsis
IL-1 receptor antagonist

Mortality at 28 days

Placebo
IL-1ra

696 total patients
No improvement in survival

Opal Crit Care Med 1997;25:1115
TNF inhibitors may increase Risk of Infection

- Patients with rheumatoid arthritis evaluated
- 144 publications reviewed
- Trimmed to 9 studies
- 5014 patients
- Careful review and extraction of the data

*JAMA 2006:2275 Bongrartz et al*
Risk of Infection with α-TNF Ab

Odds Ratio: 2.0 (1.3-3.1)

Individual Publications

Combined Results

JAMA 2006:2275 Bongrartz et al
Central Hypothesis

Not everyone is the same
Not All People Are Identical

Not All Septic Patients Are Identical
Outline for the Sepsis Talk

SIRS – Sepsis - Cytokines

Real sepsis – not endotoxemia

Legitimate animal model of sepsis

6@6, Six at Six

Early deaths, late deaths SIRS → CARS

Multiplexing for more data

Using the changes to alter therapy
What about real patients?
Patient with Acute Sepsis

- 24 year old male familial adenomatous polyposis
- 5/27 Proctocolectomy, pancreatitis post-op, also hyponatremia
- 7/24 Admitted to an outside hospital, CT scan shows dilated loops of bowel
- Possible stricture, residual polyps, doing well
Patient with Acute Sepsis, cont’d

• 7/29  Acute abdominal pain
  – X-ray shows free air
  – Emergent exploration – necrotic bowel, free stool in abdomen
  – Rx with pressors, antibiotics, aggressive fluid support

• 7/30  Cardiac arrest, resusciated

• 7/31 1:30AM Pronounced Dead
Graphic Photos to Follow
Subarachnoid Hemorrhage
Profile of “Typical Septic Patients”
27 patients
Hotchkiss J. Immun. 2001, 166:6952

• Age 62, range 18 to 92
• Days septic 12.6, range 1 to 77
• Co-Morbid Conditions
  – Renal failure with hemodialysis 10%
  – Diabetes – 10%
  – Liver disease – 10%
  – Heart disease 10%
Sepsis Therapy

- Antibiotic Therapy
- Fluid Resuscitation
- Organ support
  - Dialysis
  - Ventilator
  - Blood replacement

Other modulators are **ADDED TO STANDARD RX**
Failed Sepsis Therapies

<table>
<thead>
<tr>
<th>Treatment Type</th>
<th>Example</th>
</tr>
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<tbody>
<tr>
<td>Glucocorticoids</td>
<td>Ibuprofen (COX inhibitor)</td>
</tr>
<tr>
<td>α-endotoxin antibodies</td>
<td>PAF antagonist</td>
</tr>
<tr>
<td>Bradykinin antagonist</td>
<td>IL-1 ra</td>
</tr>
<tr>
<td>α-TNF antibodies</td>
<td>TNF-SR</td>
</tr>
</tbody>
</table>

What has worked?
Activated Protein C

Significant ↑ in survival

Activated Protein C

Stock price of Eli Lilly, June - Sep 2000

- Sepsis trials reported in August
- Prozac off patent early in September
aPC for Adults with Severe Sepsis and Low Risk of Death

*NEJM September 29, 2005 353:1332
Abraham et. al.*

Severe Sepsis: sepsis induced dysfunction of at least 1 organ
Low risk of death: APACHE < 25 or single organ failure

Required by FDA after post-hoc data analysis
2640 patients enrolled
Study terminated early

Mortality
Placebo 17.0%
aPC 18.5%
Increased Incidence of Bleeding Events

![Bar chart showing increased incidence of bleeding events during infusion.]

- **Serious Bleeding Events Day 1-6 during infusion**
  - Placebo: 15
  - aPC: 35

- **Serious Bleeding Events Day 1-28**
  - Placebo: 20
  - aPC: 60

- **Bleeding Events Leading to Transfusion**
  - Placebo: 10
  - aPC: 90

*aPC should not be used for septic patients with a low risk of death*
Outline for the Sepsis Talk

SIRS – Sepsis - Cytokines
Real sepsis – not endotoxemia
Legitimate animal model of sepsis
6@6, Six at Six
Early deaths, late deaths SIRS→CARS
Multiplexing for more data
Using the changes to alter therapy
Lipopolysaccharide
Not even close

- Lethal LPS
- Non-Lethal LPS
- Lethal CLP
- Non-Lethal CLP

Collect 20 \( \mu l \) blood over first 24 hours
A Better Model of Sepsis
Cecal Ligation and Puncture
Standard CLP Protocol

- Isoflurane Anesthesia
- Fluid resuscitation at the time of surgery
- Analgesia (buprenorphine)
- Antibiotics twice a day x 5 days
- Fluid resuscitation twice a day x 5 days
- Repeated peripheral blood sampling 20 µl
Steps in CLP

1) Open skin and peritoneum
2) Exteriorize Cecum

3) Ligate below ileocecal valve
4) Puncture twice
5) Close peritoneal cavity, running stitch or interrupted sutures

6) Insert minimetters subcutaneously
7) Close skin with wound glue
Ebong, S. *Infection & Immunity* 1999:67 pg 6603
### Temperature Profile

<table>
<thead>
<tr>
<th>Time (Hrs)</th>
<th>Temperature (°C)</th>
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<tr>
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<td>25</td>
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<tr>
<td>24</td>
<td>30</td>
</tr>
<tr>
<td>48</td>
<td>35</td>
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</table>

- **Sham**
- **25G**
- **21G**
- **18G**

* = p < 0.05
Gross motor activity and return to diurnal rhythm.
Survival proportions

Percent survival
day

- treated
- not treated

*p=0.0038

Treatment: Imipenem 25 mg/kg in LR with D5W twice/day x 5 days
Outline for the Sepsis Talk

SIRS – Sepsis - Cytokines
Real sepsis – not endotoxemia
Legitimate animal model of sepsis
6@6, Six at Six
Early deaths, late deaths SIRS→CARS
Multiplexing for more data
Using the changes to alter therapy
Overall Survival 21 G CLP

N = 69
50% mortality – what predicts?
Interleukin 6

? Biomarker for mortality

Over 30 papers show:

\[ \uparrow \text{IL6} = \downarrow \text{Survival in human septic patients} \]

Experiment, sacrifice mice at different time points after CLP of increasing lethality
Collect peritoneal fluid and plasma
Local vs Systemic IL-6 after CLP

![Graph showing the relationship between Plasma IL-6 and Peritoneal IL-6 levels. The x-axis represents Plasma IL-6 (ng/ml) ranging from $10^2$ to $10^7$, and the y-axis represents Peritoneal IL-6 (ng/ml) ranging from $10^{-1}$ to $10^9$. The data points are scattered across the graph, indicating a positive correlation.]
Objective

• Using the cecal ligation and puncture model of sepsis, can we define parameters which will predict outcome?
• Can these parameters be defined in sufficient time to initiate a therapeutic intervention?
Plasma IL-6
6 h after CLP

IL-6 AT 6 HRS

P = <.0001

IL-6 AT 6 HRS

STATUS AT 72 HRS

Shock, 2002:17 pg 463
Plasma IL-6 at 6 hours

Prediction of mortality at 3 days after CLP

Sensitivity = 91%
Specificity = 90%
IL-6 Knockout Mice

- C57 BL\6 background (previous work was BALB\c or ICR)
- Complete lack of IL-6 production
- CLP protocol used, with varying needle sizes

Infect Immun 2005:73 pg 2751
21 Gauge X 2Punctures

- KNOCKOUT n=9
- WILD TYPE n=9

p = .96

25 Gauge X 2Punctures

- KNOCKOUT n=18
- WILD TYPE n=18

p = .40

25 Gauge X 1Puncture

- KNOCKOUT n=34
- WILD TYPE n=37

p = .44

Sham

- KNOCKOUT n=9
- WILD TYPE n=9

p = 1
Body Temperature

A

Degrees Celsius

0 2 4 6 8 10 12 14 16 18 20 22 24

KNOCK OUT

WILD TYPE

Hours

B

Degrees Celsius

2 8 12 20

WILD TYPE

KNOCK OUTS

* * * * *

Hours
## Scorecard

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<tr>
<th></th>
<th>CLP</th>
<th>Sepsis</th>
</tr>
</thead>
<tbody>
<tr>
<td>28 day mortality</td>
<td>60%</td>
<td>40%</td>
</tr>
<tr>
<td>Responds to Rx</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Effect of Age</td>
<td>↑ Death</td>
<td>↑ Death</td>
</tr>
<tr>
<td>Source of infection</td>
<td>Peritoneum</td>
<td>Lung</td>
</tr>
<tr>
<td>Weight Change</td>
<td>↑ = death</td>
<td>↑ = death</td>
</tr>
<tr>
<td>Failed α-TNF</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Malaise</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Fever</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>IL-6 predicts death</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>↑ In death</td>
<td>↓ In death</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>↓ In death</td>
<td>↓ In death</td>
</tr>
</tbody>
</table>
Recap before we get lost

- Sepsis is bad for you and your next of kin
- Heterogeneity exists in the individual response to sepsis
- CLP reproduces many of the features of sepsis
- Early inflammatory markers predict early deaths
Outline for the Sepsis Talk

SIRS – Sepsis - Cytokines
Real sepsis – not endotoxemia
Legitimate animal model of sepsis
6@6, Six at Six
Early deaths, late deaths SIRS→CARS
Multiplexing for more data
Using the changes to alter therapy
Why do Septic Patients Die?

- Too Much Inflammation
  - Need to cool things down

- Too Little Inflammation
  - Need to heat things up

Hypothesis: The cause of death is different in early (5 days) vs late sepsis
Test the SIRS ⇒ CARS

• Is there evidence for this hypothesis
  – CLP performed on ICR mice
  – Necrotic cecum resected on day 3
  – Routine parameters monitored for 28 days
Plasma IL-6 Levels

- Alive for 20 days (n=30)
- Dead in 4 days (n=21)

Infect Immun 2006:74 pg 5227
Plasma IL-6 Levels

Infect Immun 2006:74 pg 5227
Plasma IL-6 Levels

Infect Immun 2006:74 pg 5227
Peritoneal Lavage

Day 15 Survivor

Day 10 Non-Survivor

Day 20 Non-Survivor

Infect Immun 2006:74 pg 5227
Peritoneal CFUs in Chronic Sepsis

Resected
Surviving
Dying

CFU/ml

10^18
10^15
10^12
10^9
10^6
10^3
1

Resected Surviving Dying

Infect Immun 2006:74 pg 5227
<table>
<thead>
<tr>
<th></th>
<th>Early</th>
<th>Late</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>Always high</td>
<td>Variably high</td>
</tr>
<tr>
<td>Bacteria</td>
<td>± present</td>
<td>Always present</td>
</tr>
</tbody>
</table>
Outline for the Sepsis Talk

SIRS – Sepsis - Cytokines
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Where do we go from here?

- IL-6 is just one of many markers
- Careful evaluation of multiple markers
- Real time evaluation to direct moment by moment therapy
Multiplex Immunoassay

- Attempt to create a protein chip to quantitate inflammatory mediators
- Essentially a sandwich ELISA on a chip
- Initial trials with 16 cytokines

Why not beads?

Cost

Cost

Cost

Cost

Cost

Shock 2004:21 pg 26
Proteomics 2005:5 pg 4712
J Immunol 2005:175 pg 3282
J Immunol 2007:179 pg 623
Principle of Protein Chip

1) Block
2) Add sample
3) Detection Antibody
4) Streptavidin Coupled to Fluorochrome Cy5
5) Read fluorescence
96 well plate single well

Antibodies spotted in quadruplicate

Location of the spot indicates what is being measured

IL-6  TNF
Correlation Between I. ELISA & Micro array

R² = 1.0
Cytokines Presently on Protein Chip

- IL-1, IL-1RA, IL-1SR II,
- IL-4, IL-6, IL-8, IL-10, IFN-γ,
- MCP-1, MIP-1α, MIP-1β, RANTES,
- TNF-α, TNF-SR I, TNF- SR II,
- βNGF
Early and Late Deaths

- What are the differences in plasma biomarkers for early vs late deaths?
- Do these have sufficient predictive power?
- Is there any value added to measuring the biomarkers?
28 DAY SURVIVAL

“ACUTE DEATHS”

39 DIED

“CHRONIC DEATHS”

17 DIED

Percent survival
days after clp

Survival at day 5 = 57%
Survival at day 14 = 49%
Survival at day 28 = 38%

n=90
KINETIC PROFILES OF CYTOKINES IN ACUTE PHASE OF SEPSIS

PRO-INFLAMMATORY CYTOKINES

IL-6

TNFα

HOURS AFTER CLP

ng/ml

CHEMOKINES

KC

MIP-1

MCP-1

EOATAXIN

HOURS AFTER CLP

ng/ml

ANTI-INFLAMMATORY CYTOKINES

IL-1ra

TNF SRI

TNF SRI

HOURS AFTER CLP

ng/ml

DEAD BY DAY 5

DEAD AFTER DAY 5

LIVED 28 DAYS

bdl   BELOW DET. LIMIT
Early deaths

IL-6

IL-6 levels over time following CLP (C) compared to days 6, 24, 48, and 72. * indicates significant difference. bdl = below detectable limit.

Bar graph showing IL-6 levels in groups: Dead by day 5, Dead after day 5, Alive at day 28. The label "6 @ 6" is not clearly explained in the image.
Early deaths

**KC**

**KC**

- Early deaths
- **KC**
- HOURS AFTER CLP
- KC @ 6
- KC ng/ml
- Dead by day 5
- Dead after day 5
- Alive at day 28
Early deaths

IL-1 Receptor Antagonist

![Graph showing IL-1ra levels over time after CLP.](image)

**IL-1ra @ 6 hours**

- Dead by day 5
- Dead after day 5
- Alive at day 28
Receiver Operator Characteristic

**Good**

ROC Curve

**Better**

ROC Curve

Sensitivity

1 - Specificity

Sensitivity

1 - Specificity
Early deaths vs. late deaths:

- Early deaths: n=34
- Late deaths: n=22

Mortality at day 5: 41%
Mortality at day 28: 62%

Graphs showing:

- Percent survival over days after CLP
- MIP-2 levels over hours after CLP
- TNFα levels over hours after CLP
- TNF SRI levels over hours after CLP

*Significant differences indicated.
Mortality Prediction

ROC Area under the curve

Early deaths

Best

Not so

Good

6 h ROC

IL-6
KC
MIP-2
MCP-1
IL-1ra
IL-10
IL-1
TNF
IL-1
TNF-SR II
TNF-SR I
Early deaths

28 DAY SURVIVAL

"ACUTE DEATHS"
39 DIED

"CHRONIC DEATHS"
17 DIED

n=90

Percent survival

DAYS AFTER CLP

survival at day 5 = 57%
survival at day 14 = 49%
survival at day 28 = 38%
Predicting Mortality

Collect samples prior to death
Measure plasma biomarkers present before the subject dies
Predicting Mortality 24 hours prior to death

Early deaths

ROC
28 DAY SURVIVAL

Percent survival

DAYS AFTER CLP

"ACUTE DEATHS"
39 DIED

"CHRONIC DEATHS"
17 DIED

n=90

survival at day 5= 57%
survival at day 14= 49%
survival at day 28= 38%

Late deaths
Predicting Mortality

Collect samples prior to death
Measure plasma biomarkers present before the subject dies

Late deaths
Late deaths

Plasma Biomarkers in Chronic Sepsis
MIP-2

MIP-2 within 24h of death

- C1
- C2
- SICK

J Immunol 2007:179 pg 623
Plasma Biomarkers in Chronic Sepsis IL-1RA

Late deaths

IL-ra within 24h of death

IL-1ra-chronic sepsis

ALIVE

DEAD

n=28

n=14
Late deaths

Chronic Sepsis TNF-SRI

TNF SR I within 24h of death

pg/ml

day of death

n=28

n=14

ALIVE

DEAD

TNF SR I-chronic sepsis

n=28

n=14
Plasma Biomarkers for Mortality in Chronic Sepsis

Late deaths

AUC value

- IL-1ra
- TNF SR I
- Body W.
- MIP-2
- MCP-1
- TNFα
- IL-10
- IL-6
- HMGB-1
- TNF SR II
Common Biomarkers
Acute and Chronic Sepsis Deaths

Acute Deaths

Chronic Deaths

24 hours prior to death
ROC

IL-6
KC
MIP-2
MCP-1
IL-1ra
IL-10
TNF
IL-1
TNF-SR II
TNF-SR I

AUC value

0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0

IL-1ra
TNF SR I
Body W.
MIP-2
MCP-1
TNF α
IL-10
IL-6
HMGB-1
TNF SR II

0.0 0.5 0.6 0.7 0.8 0.9 1.0
Outline for the Sepsis Talk

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Early deaths, late deaths SIRS $\rightarrow$ CARS
Multiplexing for more data
Using the changes to alter therapy
Using IL-6 as a guide to Rx

- High levels of IL-6 predict mortality
- Measure IL-6 and use the levels to guide therapy
- Need to develop a rapid assay for IL-6
- Non-specific inhibitor – high dose glucocorticoids
1. Early Sepsis
2. Prospective stratification
Dex Therapy

No stratification
No Help

Percent survival

Days

Treated
Not Treated

n=44

No Stratification

- Treated
- Not Treated

n=44
Predicted to live

Suggestion of harm

Percent survival

Days

Predicted to Live

- Treated
- Not Treated

n=34

p=0.27

n=35
Predicted to Die
Improve survival

Predicted to Die

Treated
Not Treated

n=10
n=9
p=0.035

Days

Percent survival

1 2 3 4 5 6 7
Dex improves 28 day survival

- Predicted to Die
  - Treated
  - Not Treated

Percent survival vs. Days

- Treated: n=9
- Not Treated: n=10
Conclusions

• Sepsis is a heterogeneous disease process
• CLP is an adequate model of disease
• Individual septic patients are optimally treated with tailored, individual therapy
• Much still needs to be done
• Translational opportunities exist as we move forward
Questions
Although the yellow fever virus is believed to have originated in Africa, the first recorded outbreak was in Mexico in the seventeenth century. This was followed during the eighteenth and nineteenth centuries by numerous outbreaks in the Caribbean, Central and South America and the eastern part of the USA as far north as New York. Epidemics in more temperate regions of the western hemisphere were the result of introductions through seaports and of transport of mosquito vectors and viruses along commercial shipping routes. At the beginning of the last century, yellow fever killed thousands yearly and was the first “filterable agent” proven to be transmitted by an insect, giving birth to a whole new category of viruses: the arboviruses. The work of the US Army Commission in Cuba, including Walter Reed, William Gorgas and other coworkers, established that transmission of yellow fever virus from humans to humans was by infected *Aedes aegypti* mosquitoes. Control measures against this mosquito, along with immunization using a live attenuated virus vaccine, effectively controlled urban yellow fever in the Americas. However, the disease persisted sporadically in rural areas of both Africa and South America as a consequence of sylvatic (jungle) cycles involving monkeys and forest-dwelling mosquitoes. In rural areas, most yellow fever infections occur in people who visit or work in the forests of Africa and South America. Periodically the virus is introduced into urban areas where the highly domesticated mosquito *Aedes aegypti* occurs. This mosquito may become infected by feeding
on a viremic person who was infected in the forest, and secondary transmission can then ensue. Urban epidemics have historically been explosive with many cases because transmission is human to human via the *Aedes aegypti* mosquito, which feeds primarily on humans.

Yellow fever illness varies from a subclinical infection to a fulminating disease terminating in death. After an incubation period of 3-10 days, there is sudden onset of fever, chills, headache and backache. Patients are usually severely ill, restless, with flushed face, swollen lips, and congested tongues and conjunctivae. Many patients suffer from nausea and vomiting and a bleeding tendency may be seen early on. A brief 1-2 day remission may occur and is quickly followed by resumption of the febrile illness. The facial edema and flushing are replaced by a dusky pallor, the gums become swollen and bleed easily, and there is a pronounced hemorrhagic tendency with hematemesis, melena and ecchymoses. In spite of a high fever, the pulse rate is slow and the blood pressure falls, resulting in renal failure with albuminuria, oliguria and anuria. Death, when it occurs, is usually within 6-7 days of onset, and is rare after 10 days of illness. The jaundice, which gives the disease its name, is generally apparent only in convalescing patients. Mortality may be as occur in 20-50%. Most patients with severe disease have leukopenia, thrombocytopenia, elevated hepatic enzymes and coagulation defects. At autopsy the organs most affected are the liver, spleen, kidneys and heart. Typically, midzonal necrosis is apparent in the liver, affecting cells around the periphery of the lobule and sparing areas around the central vein. Acidophilic necrosis is evident and Councilman inclusion bodies are usually present. Viral antigens, as detected by immunohistochemistry, are usually confined to the liver in these fatal cases.

Treatment is supportive and confined to nonspecific measures, including maintenance of fluid and electrolyte balances and replacement of any substantial amounts of blood lost.
through hemorrhage. One dose of live, attenuated 17D vaccine provides complete protection for 10 years and is notably free from reactions. Since 1937, this vaccine has protected about 44 million humans from yellow fever. However, since the late 1990s, close to 40 cases of yellow fever vaccine-associated viscerotropic disease have been reported worldwide. The risk of this adverse event is about three per million of doses administered and is highest among people over 60 years old. Virus is widely distributed in various tissues in these cases and is very distinct in cellular tropism as compared to that seen in naturally acquired disease. It is hypothesized that it may be related to be due to genetic susceptibility.

Yellow fever is caused by a flavivirus and is classified as a hemorrhagic fever virus (VHF). VHF are a special group of viruses, belonging to four different families, transmitted to humans by arthropods and rodents (Table 1). These viruses persist in nature through zoonotic cycles, although in the case of dengue and sometimes yellow fever viruses, human-to-human transmission through the bite of a mosquito vector is an important factor in disease maintenance. Other hemorrhagic fever (HF) of infectious that must be included in the differential diagnosis and excluded are malaria, rickettsial diseases, leptospirosis, shigellosis, and typhoid fever. Characteristic pathologic features of yellow fever and other viral hemorrhagic fevers are provided (Table 2). The presentation will highlight the clinical and pathologic features of yellow fever and other viral hemorrhagic fevers (VHF). The presentation will prepare pathologists to recognize yellow fever and diagnose these various infections. The differential diagnosis and anatomic pathologic approach to achieve an etiologic diagnosis of these threatening diseases will be discussed.
<table>
<thead>
<tr>
<th>VIRUS</th>
<th>DISEASE NAME</th>
<th>CASE FATALITY</th>
<th>VERTEBRATE HOST</th>
<th>ARTHROPOD VECTOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARENAVIRUSES</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Junin</td>
<td>Argentine HF</td>
<td>15-30%</td>
<td>Rodents (Calomys musculinus)</td>
<td>None</td>
</tr>
<tr>
<td>Machupo</td>
<td>Bolivian HF</td>
<td>15-30%</td>
<td>Rodents (Calomys callosus)</td>
<td>None</td>
</tr>
<tr>
<td>Guanarito</td>
<td>Venezuelan HF</td>
<td>15-30%</td>
<td>Rodents (Zygodontomys brevicauda)</td>
<td>None</td>
</tr>
<tr>
<td>Sabia</td>
<td>Brazilian HF</td>
<td>15-30%</td>
<td>Presumably an unidentified rodent</td>
<td>None</td>
</tr>
<tr>
<td>Lassa</td>
<td>Lassa fever</td>
<td>~15%</td>
<td>Rodents (Mastomys)</td>
<td>None</td>
</tr>
<tr>
<td>BUNYAVIRIDAE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rift Valley</td>
<td>Rift Valley fever</td>
<td>~ 50%</td>
<td>Vertebrates (Sheep, cattle)</td>
<td>Mosquito, Aedes</td>
</tr>
<tr>
<td>Crimean Congo</td>
<td>Crimean Congo HF</td>
<td>15-30%</td>
<td>Vertebrates (Birds, hares, large ungulates)</td>
<td>Ticks, especially Hyalomma</td>
</tr>
<tr>
<td>Hantaan, Seoul</td>
<td>Hemorrhagic fever with renal syndrome (HFRS)</td>
<td>1-15%</td>
<td>Rodents</td>
<td>None</td>
</tr>
<tr>
<td>Sin Nombre,</td>
<td>Hantavirus pulmonary syndrome (HPS)</td>
<td>50%</td>
<td>Rodents</td>
<td>None</td>
</tr>
<tr>
<td>Puumala, and</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Omsk hemorrhagic fever (OHF)</td>
<td></td>
<td>0.5-9%</td>
<td>Rodents</td>
<td>Ticks</td>
</tr>
<tr>
<td>FLAVIVIRIDAE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marburg</td>
<td>Marburg HF</td>
<td>25%</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Ebola</td>
<td>Ebola HF</td>
<td>50-90%</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>FLAVIVIRIDAE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yellow fever</td>
<td>Yellow fever</td>
<td>20%</td>
<td>Primates</td>
<td>Mosquito, especially Aedes</td>
</tr>
<tr>
<td>Dengue</td>
<td>Dengue HF, dengue shock syndrome (DHF/DSS)</td>
<td>5%</td>
<td>Primates, humans</td>
<td>Mosquito, especially Aedes aegypti</td>
</tr>
<tr>
<td>Kyasanur Forest disease (KFD)</td>
<td>KFD</td>
<td>0.5-9%</td>
<td>Rodents</td>
<td>Ticks</td>
</tr>
<tr>
<td>Omsk hemorrhagic fever (OHF)</td>
<td>OHF</td>
<td>0.5-9%</td>
<td>Rodents</td>
<td>Ticks</td>
</tr>
</tbody>
</table>
Table 3. Pathologic features in viral hemorrhagic fevers.

<table>
<thead>
<tr>
<th>DISEASE</th>
<th>PATHOLOGIC FEATURES*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argentine HF</td>
<td>Multifocal hepatocellular necrosis with minimal inflammatory response, interstitial pneumonitis, myocarditis, and lymphoid depletion. Extensive parenchymal cell and reticuloendothelial infection, more than morphologic lesions would suggest.</td>
</tr>
<tr>
<td>Bolivian HF</td>
<td>Widespread hepatocellular necrosis and hemorrhage, sometimes with midzonal distribution, minimal inflammatory response, DIC, lymphoid depletion, and encephalitis. RVF antigens in very few individual hepatocytes.</td>
</tr>
<tr>
<td>Venezuelan HF</td>
<td>Widespread hepatocellular necrosis and hemorrhage with minimal or no inflammatory cell response and lymphoid depletion. Hepatic and endothelial cell infection and damage.</td>
</tr>
<tr>
<td>Lassa fever</td>
<td>Retroperitoneal edema in severe HFRS, mild to severe renal pathologic changes. Congestion and hemorrhagic necrosis of renal medulla, right atrium of the heart, and anterior pituitary. Extensive endothelial infection mainly in renal and cardiac microvasculature.</td>
</tr>
<tr>
<td>Rift Valley fever</td>
<td>Large bilateral pleural effusions and heavy edematous lungs, mild to moderate interstitial pneumonitis, immunoblasts and atypical lymphocytes in lymphoid tissues and peripheral blood. Extensive infection of endothelial cells in pulmonary microvasculature.</td>
</tr>
<tr>
<td>Crimean Congo HF</td>
<td>Extensive and disseminated infection and necrosis in major organs such as liver, spleen, lung, kidney, skin, and gonads. Extensive hepatocellular necrosis associated with formation of characteristic intracytoplasmic viral inclusions. Lymphoid depletion, microvascular infection and injury.</td>
</tr>
<tr>
<td>Hemorrhagic fever with renal syndrome (HFRS)</td>
<td>Similar to Ebola HF</td>
</tr>
<tr>
<td>Hantavirus pulmonary syndrome (HPS)</td>
<td>Midzonal hepatocellular necrosis; minimal inflammatory response. Councilman bodies and microvesicular fatty change. Hepatocellular and Kupffer cell infection.</td>
</tr>
<tr>
<td>Ebola HF</td>
<td>Centrilobular and midzonal hepatocellular necrosis with minimal inflammatory response; Councilman bodies and microvesicular fatty change. Hyperplasia of mononuclear phagocytic cells in lymphoid tissues and atypical lymphocytes in peripheral blood. Widespread infection of mononuclear phagocytic and endothelial cells.</td>
</tr>
<tr>
<td>Marburg HF</td>
<td>Focal hepatocellular degeneration, fatty change, and necrosis. Pulmonary hemorrhage, depletion of malpighian follicles, sinus histiocytosis, erythrophagocytosis, mild myocarditis, and encephalitis.</td>
</tr>
<tr>
<td>Kyasanur Forest Disease (KFD)</td>
<td>Little known; scattered focal hemorrhage, interstitial pneumonia, and normal lymphoid tissues</td>
</tr>
</tbody>
</table>

* These features represent the more characteristic pathologic findings in the different VHF. More general findings seen to variable degrees in all HF are not listed in this table.