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


Bullet Points and keywords

- 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 (lymphotoxin)?**

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

---

**Unknown target cell**

**ICAM-1 upregulation**

**Endothelial cell**

**Effector cells**
- monocyte
- CD8+ T cell
- mLTα1β2
- sLTα1

**mTNF**

**LTβR**

**mLTα2β1**

**Effects?**

**mLTα1β2**

**mLTα2β1**

**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**

von zur Mühlen et al., J Clin Invest (2008)

---

**LIBS: detection of MRI-invisible lesions**

(A)  
(B)

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

Platelets bind to TNF-activated endothelium and potentiate its apoptosis

Platelets bind to TNF-activated endothelium and potentiate its apoptosis

2 levels of complexity

Microparticle production: membrane vesication

Vesication: microparticle (MP) production

OEDEMA
HAEMORRHAGE

Vascular Immunology Unit
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

HUVEC

resting + TNF


HBEC

A B C

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?

Are microparticles pathogenic?
MP levels are higher at the time of CM in susceptible mice

![Graph showing MP levels](image)

Enzymes controlling phospholipid movements

- **Outer leaflet**
  - PE
  - PS

- **Inner leaflet**
  - amino-phospholipid translocase
  - 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)

ABCA1 gene deletion prevents CM

- ABCA1+/+ (DBA/1)
- ABCA1−/− (DBA/1)

![Graph showing survival and parasitaemia](image)

Upon PbA infection: higher levels of plasma microparticles in ABCA1+/+ mice

- Microparticle quantitation in plasma
- Cellular origin of microparticles

![Graph showing microparticle levels](image)

References:
- 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

Microparticles from PbA-infected CM-susceptible mice are:
more procoagulant than those from PbA-infected ABCA1-KO 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 abolishes human brain endothelial vesiculation in vitro

THP-22 treatment prevents CM development

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

How?
PMP adhere to and penetrate in brain EC

MP-target interactions: 3 patterns

Some PMP are internalised in lysosomes

- "cytosolic"
- "surface"
- "compartmentalised": micropinocytosis?
Platelet MP also bind to PRBC and transfer platelet antigens to their surface.

PMP dramatically increase RBC binding to EC.

Membrane fusion Ag transfer?

Differential labelling of membrane versus cytosolic elements for the co-cultures.

1 h 30 min co-culture (after washing)

HBEC: D3 + IRBC: 3Ci
Could this transferred membrane material include *P. falciparum* antigens?

Could this transferred membrane material include *P. falciparum* antigens?

---

**Roles of microparticles during CM**

**Antigen transfer**

**Adhesion molecules**

**Adhesion + EC DAMAGE**

---

**MP as players of pathogenesis**

- Plasma TNF is high in CM
- TNF enhances endothelial MP release in vitro
  - *J Clin Invest* 1999
- High plasma MP found in patients with CM
  - *JAMA* 2004
- ABC A1 gene knock-out mice
  - Do not show a rise in MP
  - Are fully protected against the cerebral pathology
  - *Am J Pathol* 2005
- Treatment with PTTH reduces MP and prevents CM.
  - *Proc Natl Acad Sci USA* 106:1321-6, 2009

---

**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-β, not TNF-α, is required
- TNFR2 and LTβR are required

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

microparticles
- downstream and upstream events

upstream
- kinetics?
- cytoskeleton?
- rafts?

downstream
- fusion?
- internalisation
- ag transfers
- adhesiveness
- procoag.
- permeability
- Δpermeability
- novel signalling?
- trogocytosis?
- pathology?
Bacterial Sepsis

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

Daniel Remick, M.D.
Department of Pathology & Laboratory Medicine
Boston University School of Medicine
Boston Medical Center

Disclosures

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Outline for the Sepsis Talk

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SIRS - Systemic Inflammatory Response Syndrome

2 or more of the following are needed

Temperature >38° or < 36 °C
Heart rate > 90 beats/minute
Respiratory rate > 20 or PaCO₂ < 32mm Hg
WBC > 12K or < 4K/mm³, 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

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**

- Placebo
- 7.5 mg/kg
- 15 mg/kg

Mortality at 28 days: 994 total patients
No improvement in survival

*Abraham JAMA 1995;273:934*

**TNF soluble receptors TNF:Fc**

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

Mortality at 28 days: 141 total patients
Increase in mortality with therapy

*Fisher NEJM 1996;334:1697*

**Treatment of Human Sepsis**

**IL-1 receptor antagonist**

- Placebo
- IL-1ra

Mortality at 28 days: 696 total patients
No improvement in survival

*OpusCrit Care Med 1997;25:3115*

**Risk of Infection with α-TNF Ab**

- Individuals
- Publications

Combined Results

Odds Ratio: 2.0 (1.3-3.1)

*JAMA 2006;2275 Bongrartz et al*

**Central Hypothesis**

Not everyone is the same
Not All People Are Identical

Not All Septic Patients Are Identical

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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, resuscitated
• 7/31 1:30AM Pronounced Dead

Graphic Photos to Follow
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

- Glucocorticoids
- Ibuprofen (COX inhibitor)
- α-endotoxin antibodies
- PAF antagonist
- Bradykinin antagonist
- IL-1 ra
- α-TNF antibodies
- TNF-SR

What has worked?

Activated Protein C

Significant ↑ in survival

Activated Protein C

Stock price of Eli Lilly, June - Sep 2000

Sepsis trials reported
Prozac off patent early

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

aPC should not be used for septic patients with a low risk of death

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Lipopolysaccharide
Not even close

• Lethal LPS
• Non-Lethal LPS
• Lethal CLP
• Non-Lethal CLP
Collect 20 μl 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 minimeters subcutaneously
7) Close skin with wound glue

CLP Lethality

Temperature Profile

Survival proportions

**Gross motor activity and return to diurnal rhythm.**

Treatment: Imipenem 25 mg/kg in LR with D5W twice/day x 5 days
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50% mortality – what predicts?

Interleukin 6
? Biomarker for mortality
Over 30 papers show:
↑ IL6 = ↓ 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

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 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

**Scorecard**

<table>
<thead>
<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

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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**

- **Day 15 Survivor**
- **Day 10 Non-Survivor**
- **Day 20 Non-Survivor**

- **Peritoneal CFUs in Chronic Sepsis**

- **Early (5 days) vs Late Deaths**

<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**

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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?**

<table>
<thead>
<tr>
<th>Cost</th>
<th>Cost</th>
<th>Cost</th>
</tr>
</thead>
</table>

**Correlation Between I. ELISA & Micro array**

\[
R^2 = 1.0
\]

**Cytokines Presently on Protein Chip**

- IL-1, IL-1RA, IL-1SR II,
- IL-4, IL-6, IL-8, IL-10, IFN-\(\gamma\),
- MCP-1, MIP-1\(\alpha\), MIP-1\(\beta\), RANTES,
- TNF-\(\alpha\), TNF-SR I, TNF-SR II,
- \(\beta\)NGF

**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

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

KINETIC PROFILES OF CYTOKINES IN ACUTE PHASE OF SEPSIS

PRO-INFLAMMATORY CYTOKINES

CHEMOKINES

ANTI-INFLAMMATORY CYTOKINES

Early deaths

IL-6

IL-1 Receptor Antagonist

IL-1ra

Early deaths

KC

IL-6

Receiver Operator Characteristic

Good
Better
**Early deaths**

**Mortality Prediction**

ROC Area under the curve

6 h ROC

<table>
<thead>
<tr>
<th>Best</th>
<th>TNF-SR I</th>
<th>TNF-SR II</th>
<th>IL-10</th>
<th>IL-1ra</th>
<th>MCP-1</th>
<th>MIP-2</th>
<th>KC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not so</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td></td>
<td></td>
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</tbody>
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**28 DAY SURVIVAL**

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

**Late deaths**

**28 DAY SURVIVAL**

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

**Predicting Mortality**

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

**Plasma Biomarkers in Chronic Sepsis MIP-2**

J Immunol 2007:179 pg 623

**Plasma Biomarkers in Chronic Sepsis IL-1RA**

n=28 n=14

**Common Biomarkers Acute and Chronic Sepsis Deaths**

Acute Deaths

Chronic Deaths

AUC value
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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

FLOW CHART OF DECISION TREE

1. Early Sepsis
2. Prospective stratification

Dex Therapy

Predicted to live
Suggestion of harm

Predicted to Die
Improve survival
Dex improves 28 day survival

![Graph showing predicted to die days and percent survival for Treated and Not Treated groups with n=10 and n=9 respectively.]

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

The People Who Did the Work

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). VHFs 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 (VHFs). 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 1. Hemorrhagic Fever (HF) Viruses

<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><strong>ARENAVIRUSES</strong></td>
<td></td>
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<tr>
<td>Junin</td>
<td>Argentine HF</td>
<td>15-30%</td>
<td>Rodents (Calomys musculinus)</td>
<td>None</td>
</tr>
<tr>
<td>Machuipo</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><strong>BUNYAVIRIDAE</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Rift Valley fever</td>
<td>Rift Valley fever</td>
<td>~ 50%</td>
<td>Vertebrates (Sheep, cattle)</td>
<td>Mosquito, Aedes and others</td>
</tr>
<tr>
<td>Crimean Congo HF</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, Puumala, and others</td>
<td>Hemorrhagic fever with renal syndrome (HFRS)</td>
<td>1-15%</td>
<td>Rodents</td>
<td>None</td>
</tr>
<tr>
<td>Sin Nombre, Black Creek Canal, and others</td>
<td>Hantavirus pulmonary syndrome (HPS)</td>
<td>50%</td>
<td>Rodents</td>
<td>None</td>
</tr>
<tr>
<td><strong>FILOVIRIDAE</strong></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><strong>FLAVIVIRIDAE</strong></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, myocardiitidis, and lymphoid depletion. Extensive parenchymal cell and reticuloendothelial infection, more than morphologic lesions would suggest.</td>
</tr>
<tr>
<td>Bolivian HF</td>
<td></td>
</tr>
<tr>
<td>Venezuelan HF</td>
<td></td>
</tr>
<tr>
<td>Lassa fever</td>
<td></td>
</tr>
<tr>
<td>Rift Valley fever</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>Crimean Congo 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>Hemorrhagic fever with renal syndrome (HFRS)</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>Hantavirus pulmonary syndrome (HPS)</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>Ebola 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>Marburg HF</td>
<td>Similar to Ebola HF</td>
</tr>
<tr>
<td>Yellow fever</td>
<td>Midzonal hepatocellular necrosis; minimal inflammatory response. Councilman bodies and microvesicular fatty change. Hepatocellular and Kupffer cell infection.</td>
</tr>
<tr>
<td>Dengue HF/DSS</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>Kyasanur Forest Disease (KFD)</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>Omsk HF</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.