Acute Viral Hepatitis: Beyond A, B and C

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Male, 23 years old, social drinker and regular Cannabis user, who sought medical care due to a fever while working in a farm in Itambacuri (Minas Gerais, Brazil), 2 weeks from onset.

Dec 24th, 2016: Fever and Dysphagia. Treated with azithromycin.


Jan 6th: Onset of jaundice and progressive loss of consciousness.
ALT 4710 U/L
AST 5080 U/L
GGT 423 U/L

Jan 8th: ICU admission.

Jan 11th, 2017: Death.

Liver: 1,240g (1,200 – 1,600 g)

COURTESY: Renan Ribeiro, MD
Amaro Duarte, MD
HEPATITIS - Theise N in Robbins and Cotran, 9th Ed, 2015

Diffuse necro-inflammation of the liver, heterogeneous distribution. Caused by infection by hepatotropic viruses, by other viruses leading to a liver inflammation in the context of systemic infection, or due to autoimmunity, drugs or toxins.

**ACUTE HEPATITIS:**
Necro-inflammation more intense in the parenchyma usually more in acinar zone 3.

**CHRONIC HEPATITIS:**
Inflammation more relevant at the portal tract and at the porto-parenchymal interface (acinar zone 1).

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**Role of the pathologist in the management of viral hepatitis**

- **To Diagnose** Acute or chronic hepatitis (contrasting with “reactive changes of the liver in systemic infections”)
- **In Acute Hepatitis:**
  - To define **architectural patterns** of lesions:
    - Acute lobular hepatitis
    - Confluent/Submassive/Massive hepatic necrosis
  - To present data which favor **specific entities**
    - Histological patterns of lesions
    - Cytopathic effects
    - Viral antigens detectable by immunohistochemistry
    - Viral nucleic acid sequences by ISH
- **Etiology:** Multi-disciplinary approach:
  - Serology – detection of viral antigens or patient antibodies
  - Molecular virology

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**IN SITU HYBRIDIZATION**

**EBV – EBER**

Herpes Simplex hepatitis
Direct cytopathic effects

Cytomegalovirus
Immunohistochemistry

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**ACUTE VIRAL HEPATITIS**

**ETIOLOGY**

<table>
<thead>
<tr>
<th></th>
<th>INDIA, 2015</th>
<th>USA, 2014</th>
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</thead>
<tbody>
<tr>
<td>(n=206*)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAV</td>
<td>17.5%</td>
<td>19.9%</td>
</tr>
<tr>
<td>HBV</td>
<td>8.7%</td>
<td>44.8%</td>
</tr>
<tr>
<td>HCV</td>
<td>0.5%</td>
<td>35.3%</td>
</tr>
<tr>
<td>HDV</td>
<td>0</td>
<td>no</td>
</tr>
<tr>
<td>HEV</td>
<td>46.1%</td>
<td>no</td>
</tr>
<tr>
<td>MIX</td>
<td>13.1%</td>
<td>no</td>
</tr>
<tr>
<td>OTHERS</td>
<td>14.1% (CMV, EBV)</td>
<td>no</td>
</tr>
</tbody>
</table>

*Gupta E... Indian J Gastro. 2015;34:448
PATTERNS OF LESIONS

<table>
<thead>
<tr>
<th>Patterns of Acute Viral Hepatitis</th>
<th>Differential Diagnosis</th>
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</thead>
<tbody>
<tr>
<td>Lobular hepatitis</td>
<td>Hepatitis A, hepatitis B, hepatitis C</td>
</tr>
<tr>
<td>Confluent necrosis</td>
<td>Hepatitis A, hepatitis B, hepatitis C</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>Hepatitis A, hepatitis B, hepatitis C</td>
</tr>
<tr>
<td>Spotty necrosis</td>
<td>Hepatitis A, hepatitis B, hepatitis C</td>
</tr>
</tbody>
</table>


ACUTE “LOBULAR” VIRAL HEPATITIS

APOPTOSIS
SPOTTY NECROSIS

Acute Viral Hepatitis 
“CONFLUENT NECROSIS”


Fulminant Hepatic Failure - Submassive Hepatic Necrosis

SUBMASSIVE HEPATIC NECROSIS


ACUTE VIRAL HEPATITIS
“MASSIVE HEPATIC NECROSIS”
Although nowadays etiology is defined by serology or by molecular virology, histological patterns serve as “hints” for the causal agent.

ACUTE “LOBULAR” VIRAL HEPATITIS
Mononucleosis-like (EBV/CMV)
sinusoidal lymphocytosis

HCV: acute hepatitis

ACUTE “LOBULAR” VIRAL HEPATITIS
Microabscess - Cytomegalovirus

HCV: lobular pattern of acute hepatitis

IN SITU HYBRIDIZATION
HCV RNA
Qian Z, Guerrero R, Plummer T, Alves VA, Lloyd R
Diagn Mol Pathol 2004; 13: 9-14

IMMUNOHISTOCHEMISTRY
HCV Core Ag (Ab Rb246)
Alves VA – Mod Path 1998 (Abst)
Liver International 2008; 28: 807-813

Table 1. Hepatitis C virus core antigen distribution in liver tissue among groups.

<table>
<thead>
<tr>
<th>Time post-opa*</th>
<th>Severe recurrence group 1 (n=48)</th>
<th>Mild recurrence group 2 (n=46)</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Early post-transplantation follow-up (n=1)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Late post-transplantation follow-up (n=1)</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

*Grade 0: meaning no HCV core Ag detected; grade 1: ≤ 25% of the hepatocytes in the section; grade 2: 26-50%; and grade 3 > 50%.

**P=0.01.

Liver International 2008; 28: 807-813

Ground glass hepatocytes and HBsAg
Chronic HBV Infection

HEPATITIS DELTA
HDV + HBV COINFECTION OR SUPERINFECTION
Genetic Diversity of HDV

Fulminant hepatic failure in northern Brazil: morphological, immunohistochemical and pathogenic aspects of Lábrea hepatitis and yellow fever

Portal Phlebitis + Portal and Acinar Fibrosis / Elastic Fibers

Lábrea Hepatitis = acute HDV on chronic HBV
**Classical Morula (spider) Cells**

<table>
<thead>
<tr>
<th></th>
<th>YF</th>
<th>Lábrea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morula (spider cells)</td>
<td>0 + ++ +++</td>
<td>0 + ++ +++</td>
</tr>
</tbody>
</table>

Lábrea Hepatitis = HDV + HBV

**Delta Antigen – HDV Ag**

![Delta Antigen](image)

Courtesy: Luis Freitas, MD; Juliana Freitas, MD

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**Labrea Fulminant HDV+HBV Hepatitis:**

- Pan-acinar lytic hepatocellular necrosis,
- Classical morula cells
- Portal and centrilobular phlebitis.
- Portal and acinar fibrosis (acute HDV on chronic HBV)

**Hepatitis A**

Predominant lobular features

- Spotty necrosis and many apoptotic bodies.

**Portal infiltrate may be impressive**

- Plasma cells and lymphocytes
- Frequently “spill over” into the limiting plate.

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Acute hepatitis A, 2nd peak
Drebber U - 2016

**Hepatitis A**

Predominant lobular features

- Spotty necrosis and many apoptotic bodies.

**Portal infiltrate may be impressive**

- Plasma cells and lymphocytes
- Frequently “spill over” into the limiting plate.

Serological positivity for HAV IgM is important for the differential diagnosis with chronic interface hepatitis, especially in cases with prolonged or relapsing disease, which may last for more than 6 months.

ACUTE VIRAL HEPATITIS
"MASSIVE HEPATIC NECROSIS"


Histopathology of Acute and Chronic Hepatitis E

**Acute**

- Lobular Pattern of acute hepatitis
- Diffuse cellular portal and lobular inflammation
- Mixed lymphocytic and neutrophil cholangiolitis
- Bile ductular proliferation
- Cholestasis

Drebber U., 2016

ACUTE VIRAL LIVER FAILURE IN USA, 2014
CAUSES OF DEATH
(89 cases reported)

<table>
<thead>
<tr>
<th>Virus</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAV</td>
<td>76</td>
</tr>
<tr>
<td>HBV</td>
<td>13 *</td>
</tr>
<tr>
<td>HCV</td>
<td>0 *</td>
</tr>
<tr>
<td>HDV</td>
<td>no</td>
</tr>
<tr>
<td>HEV</td>
<td>no **</td>
</tr>
</tbody>
</table>

* Death due to CHRONIC HEPATITIS (CIRRHOSIS; HCC)
HBV: 1,843
HCV: 19,659
** Other countries: 0.1 – 4.0 % of infections with HEV lead to death

One in every two porks is infected with hepatitis E virus. HEV spread in Germany is attributed to raw porcine meat.


Histopathology of Acute and Chronic Hepatitis E

Acute

- Lobular Pattern of acute hepatitis
- Diffuse cellular portal and lobular inflammation
- Mixed lymphocytic and neutrophil cholangiolitis
- Bile ductular proliferation
- Cholestasis

Drebber U., 2016

Acute hepatitis E.
(B) Enlarged portal tract with lymphocytes and some PMN + spotty necrosis in the lobule
(C) Spotty necrosis, apoptosis + lymphocytes, Kupffer cells and few polymorphnuclear

Detection of HEV in Liver Tissue
RT-PCR
In situ Hybridization
Immunohistochemistry
– Monoclonal Antibodies: pORF2 / pORF3

Histopathology of Acute and Chronic Hepatitis E
Submassive Acute Hepatitis
More frequent in Pregnant Women

Chronic
• Portal-Periportal Pattern of chronic hepatitis
• Portal inflammation and fibrosis
• Conspicuous cholangitis

Male, 23 years old, social drinker and regular Cannabis user, who sought medical care due to a fever while working in a farm in Itambacuri (Minas Gerais, Brazil),
2 weeks from onset
Dec 24th, 2016: Fever and Odynophagia Treated with azithromycin
Jan 8th: Onset of jaundice and altered level of consciousness
ALT 4710 U/L
AST 5080 U/L
GGT 423 U/L
19 days from onset
Jan 11th, 2017: Death

Jan 3rd, 2017: liquid diarrhea and malaise
Jan 6th: Onset of jaundice and altered level of consciousness

Mid-dec: Trip to Itambacuri
Dec 24th, 2016:
Fever and Odynophagia
Treated with azithromycin

Viral hemorrhagic fevers (VHFs)

Severe systemic infections characterized by fever and hemorrhage, caused by four distinct families of small, lipid-enveloped RNA viruses

- Animal reservoirs - arthropods, rodents, ruminants and primates
- Human infection next to these animals’ habitat, mostly in the Tropics
- Human-to-human transmission or airborne infections occasionally detected.

Filoviridae: Ebola virus, Marburg virus, Carvavirus
Bunyaviridae: Hanta virus, Phlebovirus type species: Rift Valley fever virus, Nairovirus, Orthobunyavirus, Toxoplasma
Arenaviridae: Guanarito virus, Junin virus, Lassa virus, Lujo virus, Machupo virus, Sabia virus, Whitewater Arroyo virus
Flaviviridae: Yellow Fever virus, Dengue virus, Zika virus, tick-borne encephalitis virus, West Nile virus

Yellow Fever

- Numerous apoptotic bodies (Councilman-Rocha Lima bodies)
- Hemorrhagic hepatocyte necrosis, predominantly midzonal (zone 2)
- Non-necrotic rings of periportal and perivenular hepatocytes in zones 1 and 3
- Macrovesicular and microvesicular steatosis

Molecular Virology

- Blood (sampled in Jan/8/2017):
  - Positive for YF IgM
  - Positive for wild type YF RNA (RT-PCR / Conventional PCR)
- Liver and kidney samples from autopsy
  - Positive for wild type YF RNA (RT-PCR/Conventional PCR)
Public health officials in Brazil suspect that a yellow fever outbreak centered in Minas Gerais has resulted in 550 suspected and 72 confirmed cases, and 40 confirmed deaths. Three of the deaths have been reported in neighboring Sao Paulo state, but were reportedly contracted in the endemic area.

The Government of Brazil and the CDC recommend yellow fever vaccination for travelers to the affected areas. Affected areas include: all areas of Acre, Amapá, Amazonas, Distrito Federal (including the capital city of Brasília), Goiás, Maranhão, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Pará, Rondônia, Roraima, and Tocantins. Vaccination is also recommended for designated areas of the following states: Bahia, Pará, Piauí, Rio Grande do Sul, Santa Catarina, and São Paulo.

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- **Etiology**: Multi-disciplinary approach:
  - Serology – detection of viral antigens or patient antibodies
  - Molecular virology
Viscerotropic Disease associated with YF Vaccine

YF: Only one serotype
1936: live, attenuated (17D) vaccine, by serial passage in chicken-embryo tissue
More than 400 million people vaccinated
Long-lasting neutralizing antibodies in 99%

Viscerotropic disease:
Extensive infection by a 17D virus
Indistinguishable from wild-type yellow fever disease
60% case fatality rate

Vasconcelos, P. Lancet 2001; 358: 91
Martin M Lancet. 2001; 358: 98

Viscerotropic Disease associated with YF Vaccine

Review of the safety data of 17D YF vaccine:
Genetic stability of 17D YF vaccine,
immunological responses of healthy subjects post-vaccination and the long-term immunogenicity of 17D YF vaccines

Host factors
Genetic (genes involved in interferon responses)
Acquired (advanced age and thymectomy)

Incidence:
~ 1 / 200,000 - 400,000 vaccinations
> 60 yrs of age: 1 / 50,000 vaccinations
Serious adverse events associated with yellow fever 17DD vaccine in Brazil: a report of two cases

Vasconcelos P, ... Alves VA, ... Lancet. 2001 Jul 14;358(9276):91-97.


Icteric Hemorrhagic Fevers

Clinical presentation and lab results indicate both liver injury and extra-hepatic manifestations

High and prolonged fever, variable levels of serum transaminase elevation, multiple organ dysfunction

Tropism toward endothelial cells

Clinically, vascular lesions may lead to petechial rash and/or internal bleeding

Fatal forms present with massive hemorrhage, shock and disseminated intravascular coagulation

Differential Diagnosis

- LEPTOSPIROSIS
- HANTAVIRUS
- DENGUE FEVER
- YELLOW FEVER
- SPOTTY FEVERS (RICKETTSIOSES)

Zika Cases Reported in the United States (2015-2017)

Lab-confirmed Zika virus disease cases reporting ArboNET by state or territory

As of February 15, 2017

Symptomatic cases: 5017

CDC Zika Virus Home – visited Feb 18th, 2017

Areas with Zika

All Countries & Territories with Active Zika Virus Transmission

CDC Zika Virus Home for Healthcare Providers

Visited on Feb 18, 2017

Zika virus is a single-stranded RNA virus of the Flaviviridae family, genus Flavivirus. Transmitted primarily through the bite of infected Aedes aegypti and Aedes albopictus. Primates are likely the main reservoirs of the virus, and anthropogenic (human-to-vector-to-human) transmission occurs during outbreaks. Perinatal, in utero, and possible sexual and transfusion transmission reported. Zika virus RNA has been identified in asymptomatic blood donors.

Clinical Signs & Symptoms

Most: asymptomatic

Acute fever, maculopapular rash, arthralgia, myalgia, headache, conjunctivitis

Usually mild with symptoms lasting for several days to a week

Severe disease is uncommon. May provoke Guillain-Barré syndrome

CDC concluded that Zika virus infection during pregnancy is a cause of microcephaly and other severe fetal brain defects.

Laboratory diagnosis Blood detection of virus, viral nucleic acid, or virus-specific immunoglobulin M and neutralizing antibodies.

www.cdc.gov/chikungunya/geo/united-states-2016

• Chikungunya virus disease became a nationally notifiable condition in 2015.
• As of January 17, 2017, a total of 175 chikungunya virus disease cases with illness onset in 2016 have been reported to ArboNET from 37 U.S. states.
• All reported cases occurred in travelers returning from affected areas.
• No locally-transmitted cases have been reported from U.S. states.
• A total of 171 chikungunya virus disease cases with illness onset in 2016 have been reported to ArboNET from U.S.
• To date, 170 locally-acquired cases and 1 travel-associated case have been reported from Puerto Rico.
**Dengue Virus**

WHO: 100 million people infected, mostly in tropical and subtropical regions. Transmission by mosquitoes, mainly A. aegypti. Potentially severe hemorrhagic forms affected 250,000 people. Many outbreaks of Dengue infection in Latin America in the last decade. Brazil: 8,440,253 cases and 3058 fatal cases between 2000-2010. USA (CDC): Texas outbreak in 2005. Small outbreak occurred in Hawaii in 2001. Kay M. Tomasiel... Sherif R. Zaki... PLOs Neglected Tropical Diseases - Oct 11, 2016. Enhanced Surveillance for Fatal Dengue-Like Acute Falciparum Illness in Puerto Rico, 2010-2012. This surveillance system found the dengue mortality rate was 1.05 per 100,000 Puerto Rico residents in 2010, the highest rate ever detected.

**Liver pathology of Dengue fever**

Asymptomatic elevation of aminotransferases/ Acute hepatitis / Acute Liver Failure. Apoptosis triggered by a combination of viral cytopathic effect, hypoxic mitochondrial dysfunction, local immune response and accelerated endoplasmic reticulum stress. Midzonal and Centrilobular Necrosis caused by the immune response or from viral tropism and hypoxia combined.

**Differential diagnosis with Yellow Fever:**
- Usually more slowly progressive (several weeks)
- Less intense fatty change and Less intense necrosis
- Injury is more intense in zone 3

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**ACUTE VIRAL HEPATITIS - REGENERATION**


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**Ebola and Marburg Viruses**

Person-to-person transmission led, in several African countries, to very severe outbreaks having bats as major reservoirs. A small number of EVD cases were diagnosed in Europe and the United States during the present outbreak, most of them associated with travel to West Africa. Clinical manifestations included high fever and massive hemorrhage, shock and disseminated intravascular coagulation.

**Histopathology of the Liver**

Similar to other VHF's with hepatitis pattern without cholestasis.Main findings: hepatocellular necrosis, from spotty to confluent + minimal inflammation. Characteristic intracytoplasmic viral inclusions within hepatocytes of patients dying with EVD (less frequent in Marburg viruses). Mild to moderate microvesicular steatosis, Kupffer cell hypertrophy and hyperplasia.
Tissue and cellular tropism, pathology and pathogenesis of Ebola and Marburg viruses
Rosecelis Brasil Martines ... Sherif Zaki Journal of Pathology 2015; 235:153 - 174

Severe hemorrhagic fevers, with case fatality rates in the range 25–90%. Although most characteristic histopathological findings are seen in the liver, the findings overlap with many other viral and non-viral hemorrhagic diseases. The pathogenesis of filovirus infections is complex and involves activation of the mononuclear phagocytic system, with release of pro-inflammatory cytokines, chemokines and growth factors, endothelial dysfunction, alterations of the innate and adaptive immune systems, direct organ and endothelial damage from unrestricted viral replication late in infection, and coagulopathy. Many questions remain unanswered.

Histopathological features are similar in Marburg virus and Ebola virus infections, with necrosis seen in many organs, including liver, spleen, kidney and gonads, the gastrointestinal tract and endocardium

Liver

Hepatocyte necrosis ranges from focal to widespread, often with minimal inflammation.
Mild to moderate small-droplet steatosis and Kupffer cell hyperplasia
EBOV: Portal tracts usually exhibit extensive karyorrhexis and mononuclear infiltrate.
Hepatocytes: Characteristic intracellular eosinophilic and filamentous or oval virus inclusions, predominantly in periportal zones and surrounding areas of necrosis
The inclusions are usually more readily identified in EBOV infections than in MARV infections

Ebola virus infection: hepatocellular necrosis, sinusoidal dilatation and congestion; note numerous intracytoplasmic eosinophilic inclusions (arrow).

EM photomicrographic hepatic viral inclusions (arrow) within infected hepatocytes; note the extracellular sinusoidal virus particles (arrowhead);
Lung:
Congestion. Absence of inflammation.
Intra-alveolar macrophage with a
cytoplasmic eosinophilic inclusion (arrow).
Ebola virus antigens in intra-alveolar
macrophages and interstitium;
(D) In situ hybridization: Evidence of viral
replication in intra-alveolar macrophages.
(E) EM photomicrograph, showing Ebola
virus inclusion (arrow) in an intra-alveolar
macrophage, suggesting that viral
replication occurs within the macrophage.
(F) EM: Extracellular Ebola virus particles
(arrowhead) floating in the alveolar space.

ACUTE “LOBULAR” VIRAL
HEPATITIS

Regeneration from Progenitor Cell

Michalopoulos GK, Khan Z. Liver Stem Cells: Experimental
Findings and Implications for Human Liver Disease. Gastroenterology.
Acute hepatitis E.

(A) Expanded portal tract, dense lymphocytic infiltrate + mild cholangitis

(D) Foci of spotty necrosis, ballooning of hepatocytes + infiltrates with lymphocytes and PMN