

Diagnosis, prevention and management of HEV infection

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HEV is omnipresent

Developing countries

Asia – Africa – South America

Endemic & epidemic infection
Sporadic cases

Faecally – contaminated water

Developed countries

Europe – North America - Japan

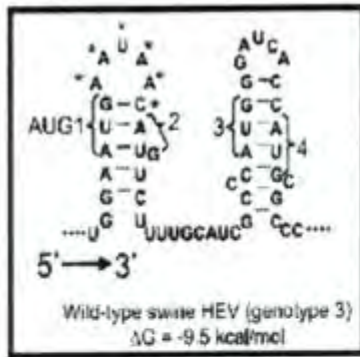
Sporadic cases
Imported infection... but also
acquired locally
Mansuy JMV 04

Zoonotic transmission from a
porcine reservoir
Meng PNAS 97

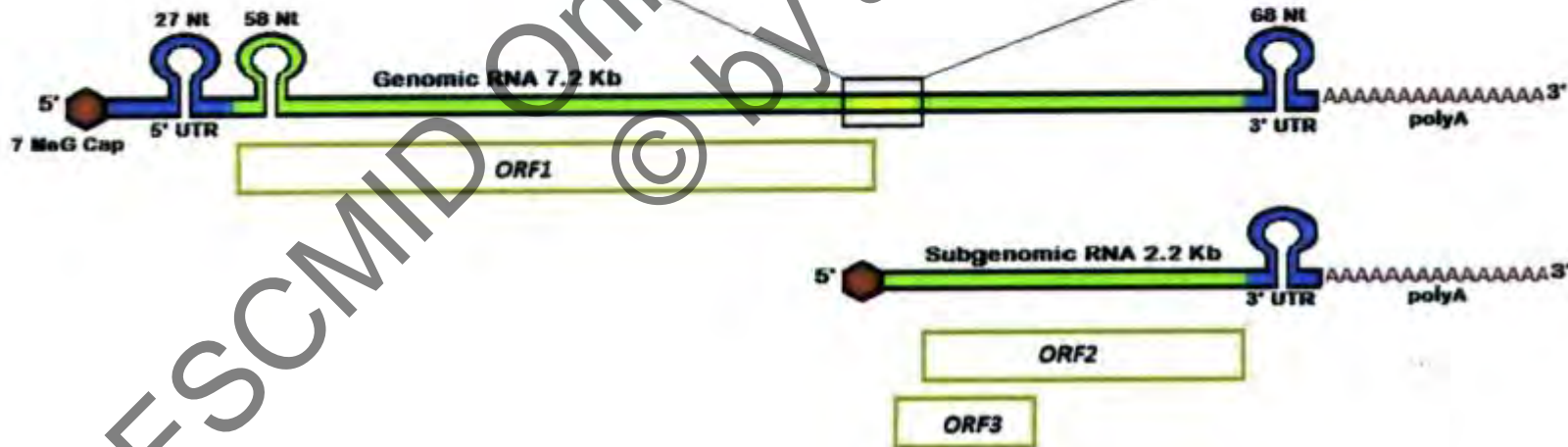
Improvements in serologic & molecular tests

HEV genome

ss (+) RNA 7.2 kb

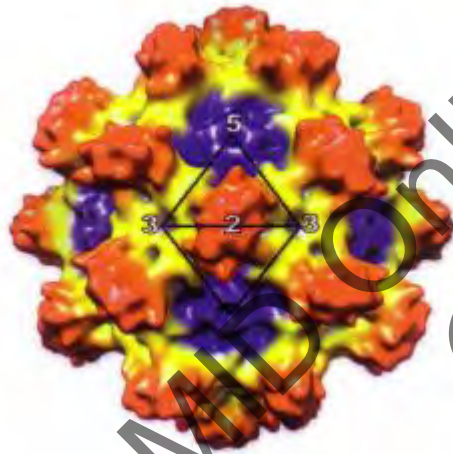


Genotype	5104	5113	5122 sgRNA	5131	5145
Genotype 1: Sar-55	GA ATG AATAAC ATG T	CTTTT	GCTGCGCCC	ATG GGTTCGCGACC	ATG CGC
Genotype 2: Mexico	ATG	ATG	ATG	ATG	ATG
Genotype 3: Swine HEV*	ATG	ATG	ATG	ATG	ATG
Genotype 4: T1	ATG	ATG	ATG	ATG	ATG

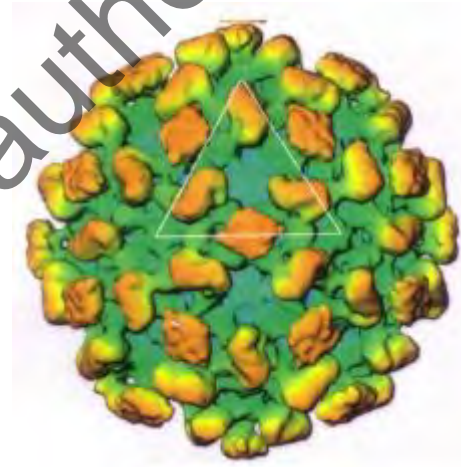


HEV capsid

- ✓ 660 aa with 3 glycosylation sites
ASN 137, 310, 562
- ✓ Expression in vitro of VLP



T = 1, 60 monomers
PORF2 Δ 111aa Nt



T = 3, 180 monomers
PORF2 Δ 13aa Nt

In vitro production of HEV

- ✓ Primary hepatocytes from non human primates
Tsarev JMV 94 ; Tam Virology 97
- ✓ PLC/PRF/5 & A549 cell lines
 - Stools : *Takahashi JCM 07 ; Tanaka JGV 07*
Lorenzo Virus Res 08 ; Tanaka JCM 09
 - Serum : *Takahashi JCM 10*
- ✓ Hep G2/C3A cell line : *Shukla PNAS 11*

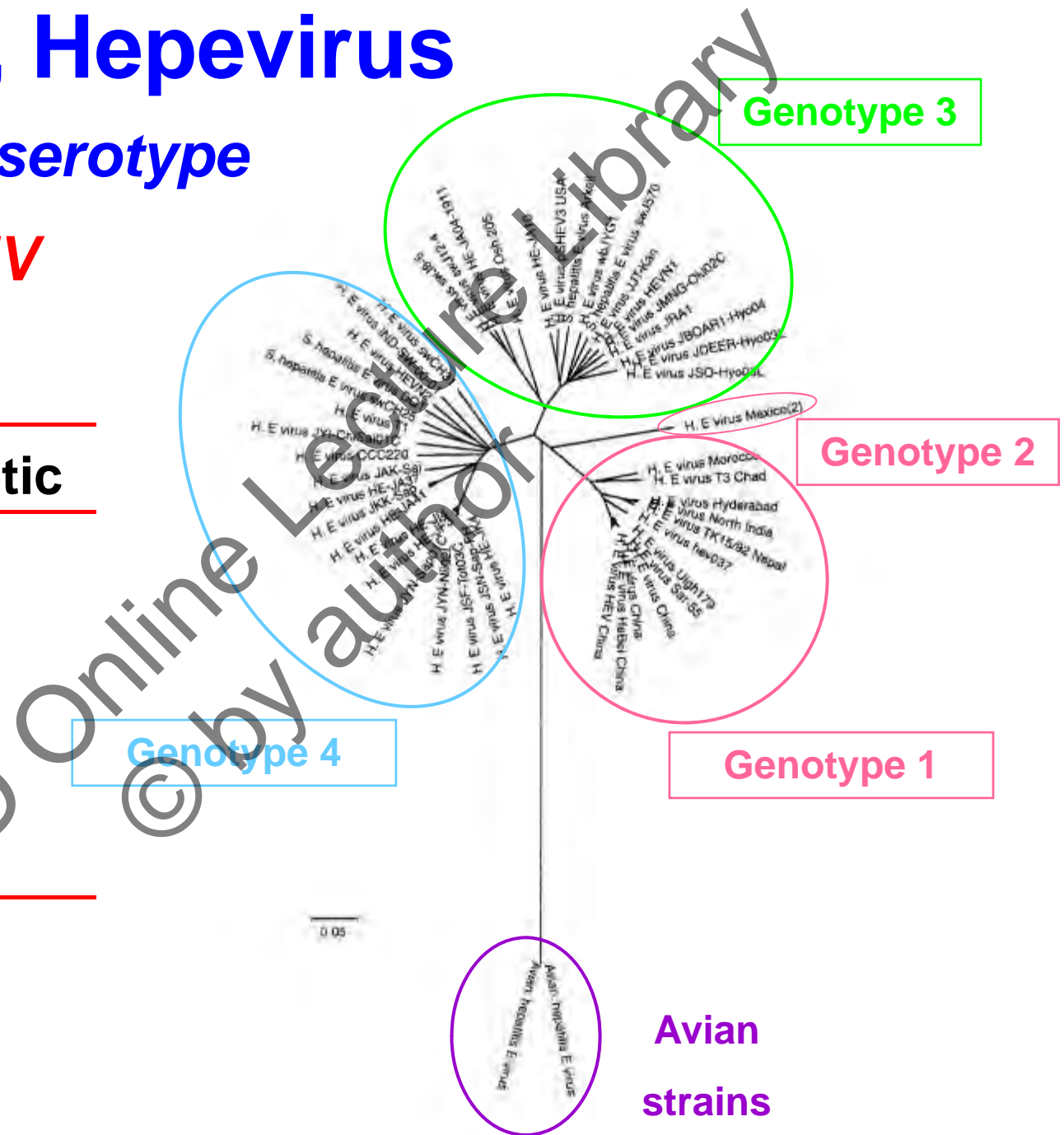
Hepeviridae, Hepevirus

Single serotype

Mammalian HEV
4 genotypes

G	Sub-G	Zoonotic
1	5	no
2	2	no
3	10	yes
4	7	yes

Avian HEV
3 genotypes



Acute hepatitis

Developing countries

Developed countries

Male

Male

Young adult 15-35 years

Adult > 55 years

Fulminant hepatitis in pregnant women

Khuroo JVH 03

Fulminant hepatitis in the context of pre-existing chronic liver disease

Dalton Lancet 07 ; Peron JVH 07

Jaundice (70 %), asthenia (40 %), fever (27 %), arthromyalgia, nausea, anorexia, abdominal pain, malaise

Chronic hepatitis E in immunocompromised patients

- ✓ **Solid-organ transplant recipients**
*Kamar NEJM 08 ; Gerolami NEJM 08 ;
Haagsma Liver Transpl 08*
- ✓ **Patients with haematological disease**
*Peron J Gastroenterol Hepatol 06 ;
Tamura Hepatol Res 07 ; Ollier Ann inter Med 09*
- ✓ **HIV infection with low CD4 cell counts < 100/mm³**
Dalton NEJM 09; Colson J Clin Virol 09 ; Kenfak EID 11

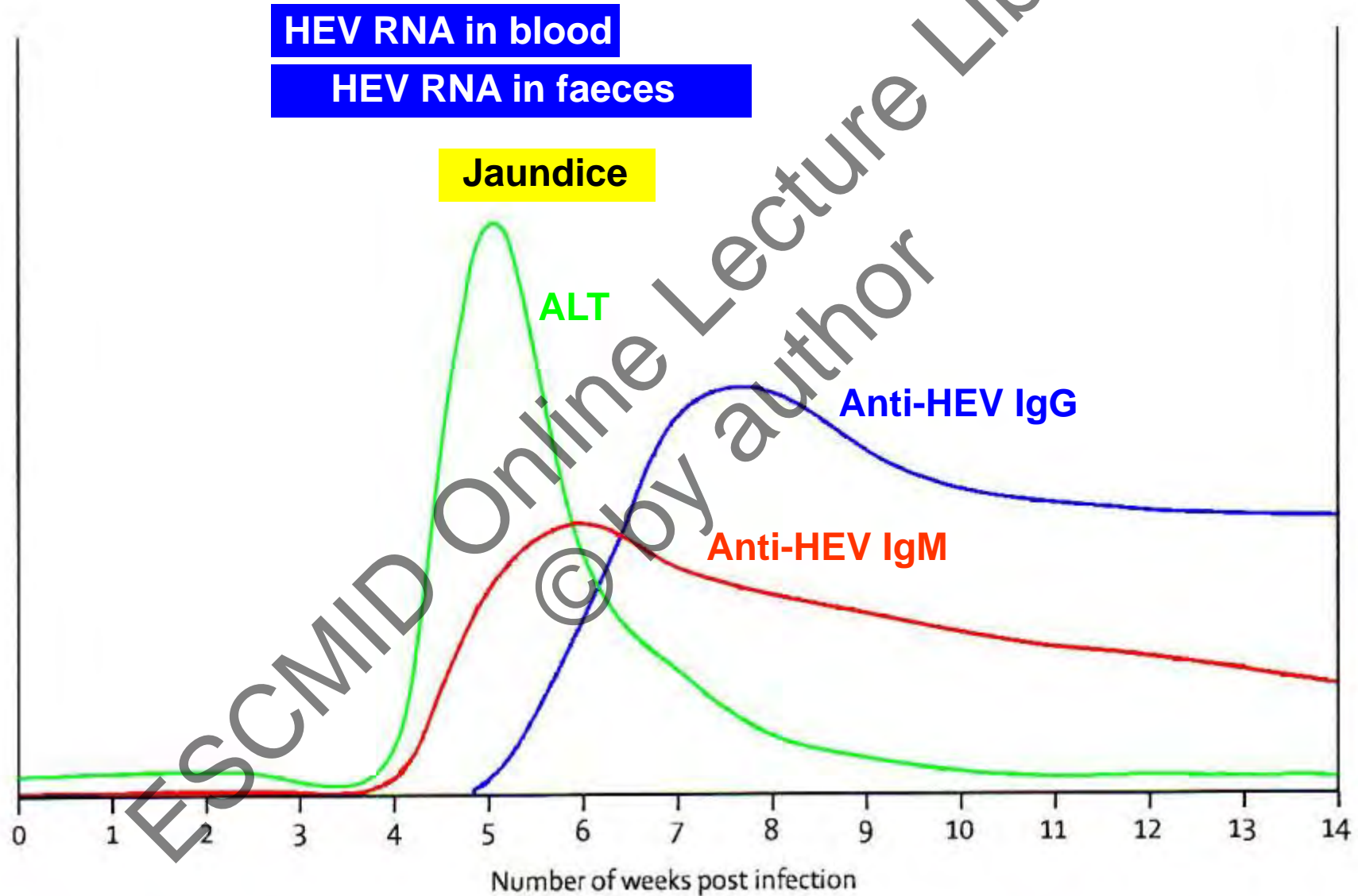
Extra-hepatic manifestations

- ✓ **Neurological complications**
*Kamar AJT 10 ; Kamar EID 11 ; Maurissen Infection 11 ;
Despierres IED 11*
- ✓ **Other complications : glomerulonephritis, acute pancreatitis
thrombocytopenia**
*Kamar Transplantation 12 ; Deniel JCV 11 ; Fourquet JCV 11
Colson JCM 08*

Unrecognised HEV in suspected drug-induced liver injury

- ✓ UK study : 6/47 (13 %) cases were due to HEV3 infection
Dalton Aliment Pharmacol Ther 07
- ✓ US study : 9/318 (3 %) cases were due to HEV3 infection
Davern Gastroenterology 11

Laboratory diagnosis



Serology

- ✓ **First line of diagnosis – commercial enzyme immunoassay & rapid immuno-chromatographic kits**
- ✓ **Based on ORF2/ORF3 peptides or recombinant antigens from HEV1**
- ✓ **Key markers :**
 - anti-HEV IgM : acute infection**
 - anti-HEV IgG : acute infection & past infection**

Anti-HEV IgM

- ✓ Six immunoassays were recently evaluated
- ✓ Sensitivity panel : 50 serum samples containing HEV RNA genotypes 1 – 4
- ✓ Specificity panel : 229 serum samples
- ✓ Study conclusions :
 - wide variations in diagnostic sensitivities, specificities & inter-assay agreement
 - limits in analytical sensitivity based on serial dilutions

Anti-HEV IgM

Test	Antigen	Sensitivity	Specificity
EIA Adaltis	Syntetic peptides G1, ORF2/3	90 %	100 %
EIA MP Diagnostics	Recombinant Ag G1, ORF2	88 %	99.5 %
Rapid immunochromatographic test - MP Diagnostics	Recombinant Ag G1, ORF2	82 %	100 %

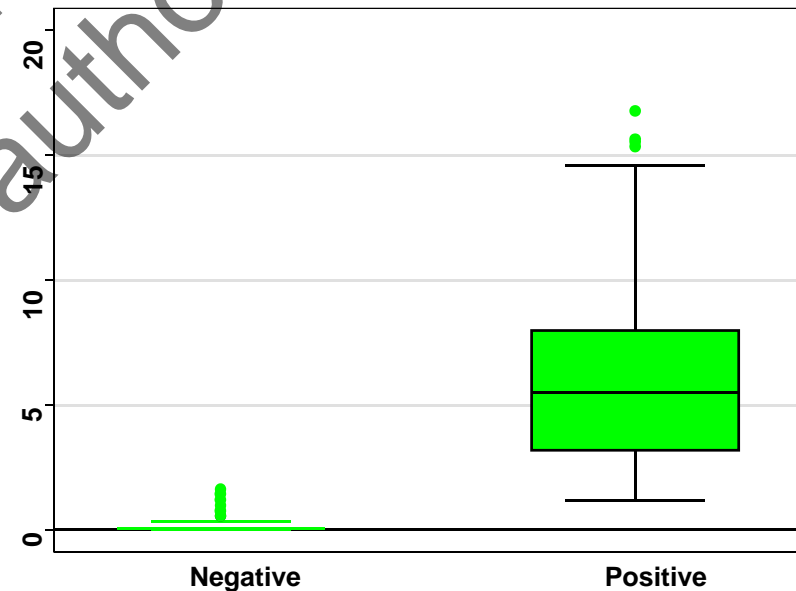
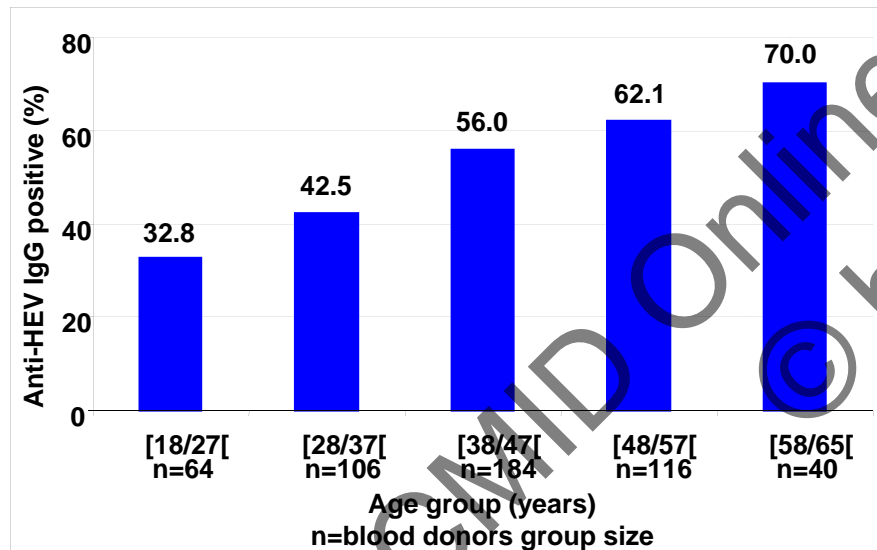
Sensitivity panel : 50 serum samples containing HEV3 RNA
Specificity panel : serum samples from blood donors

Anti-HEV IgG

- ✓ International standard available
- ✓ Commercial assays display differences in their limit of detection : 2.5 WHO units/ml vs 0.25 WHO units/ml
Bendall JMV 10
- ✓ Implications for estimating HEV seroprevalence
 - 3-4 times increase in estimates with the most sensitive assay

Blood donors in Midi-Pyrénées France

n = 512
Anti-HEV IgG : 52.5 % (Wantai) vs 16.6 % (Genlabs)



Other populations in Midi-Pyrénées France

- ✓ Patients with haematological diseases (n=88)
 - anti-HEV IgG 36.4 % (Wantai) vs 12.5 % (Adaltis)
 - Abravanel JCV 12*
- ✓ Solid-organ transplants (n=171)
 - anti-HEV IgG Wantai : 43.8 %
- ✓ Young children 2-4 years (n=188)
 - anti-HEV IgG Wantai : 3.7 %

Complementary serologic assays

- ✓ **Avidity assay : low avidity in favour of a recent infection**
Bendall JMV 08
- ✓ **Immunoblot : utility questionable for IgG due to the lack of specificity (only 66 % in one study)**
Herremans CVI 07

Molecular tests

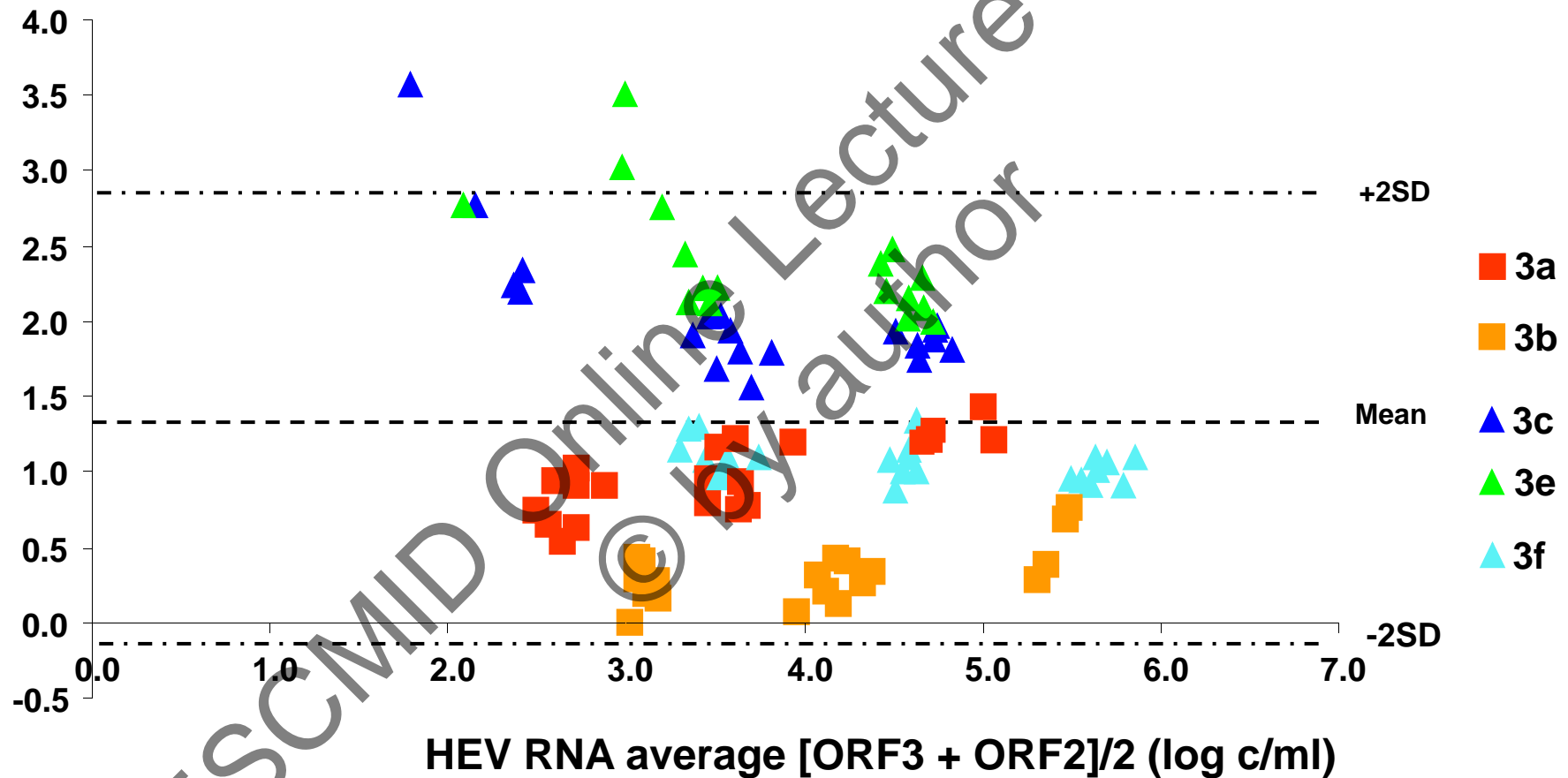
- ✓ Detection & quantification of HEV RNA in blood and stools based on real time PCR targetting ORF2 or ORF3 regions
- ✓ Limit of detection : 100-200 c/ml
Mansuy JCM 04 ; Jothikumar JMV 06 ; Gyarmati JMV 07
- ✓ Variations in assay performance in a multicentric international study *Baylis JCM 11*

Panel of 22 samples G3a, 3b, 3f, 4c
Interlaboratory variability in sensitivity
(Δ 2-3 log) and reproducibility (0.4-1 log)

Influence of HEV3 diversity

Panel of HEV genotypes 3a, 3b, 3c, 3e, 3f

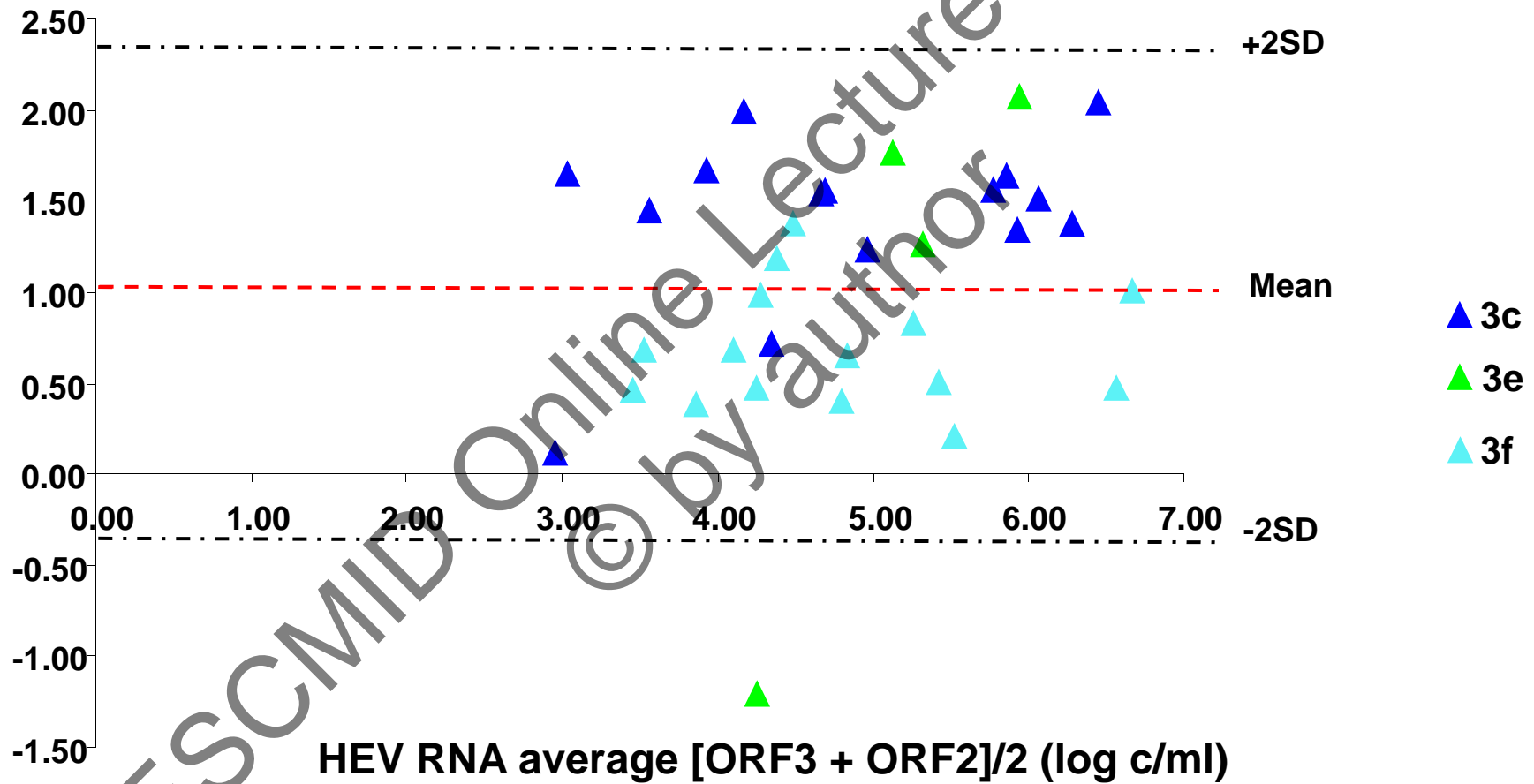
HEV RNA [ORF3 - ORF2] (log c/ml)



Influence of HEV3 diversity

34 clinical samples - HEV genotypes 3c, 3e, 3f

HEV RNA [ORF3 - ORF2] (log c/ml)



Analytical implications

- ✓ HEV diversity must be taken into account for designing the PCR primers
 - protocols based on ORF3 region provided the best performance
- ✓ The WHO international standard (G3a) recently available will be helpful for standardising detection & quantification of HEV RNA
- ✓ Validation of commercial assays in development using appropriate HEV panels is mandatory

Interest of molecular tests

- ✓ Diagnosis of acute infection in seronegative patients
 - immunocompetent patients *Mansuy JCM 04*
 - immunocompromised patients *Kamar NEJM 08*
- ✓ Diagnosis of chronic infection in immunocompromised patients
 - persistence of HEV RNA for more than 6 months
- ✓ Confirmation of HEV infection and genotyping
 - characterization of G3/4 (autochthonous zoonotic transmission) vs G1/2 (imported disease)
- ✓ Monitoring of therapeutic intervention

Solid organ transplant patients

- ✓ Longitudinal study of 700 patients followed-up for 5 years *Abravanel EID 11*
 - annual incidence : 3.2 %
 - no reactivation in anti-HEV (+)
- ✓ Transmission via the graft documented
Schlosser J Hepatol 11
- ✓ Chronic infection in 60 % of the cases, associated with tacrolimus (vs cyclosporin) and thrombocytopenia
Kamar Gastroenterology 11
- ✓ Pediatric-transplant patients *Halac Gut 11*

Patients with haematological disease

- ✓ Reactivation documented in a anti-HEV (+) patient after allogenic stem cell transplantation

Le Coutre Gut 09

- ✓ No reactivation in 32 anti-HEV (+) patients after allogenic or autologous stem cell transplantation, including 3 patients who were also anti-HEV IgM (+)

Abravanel JCV 12

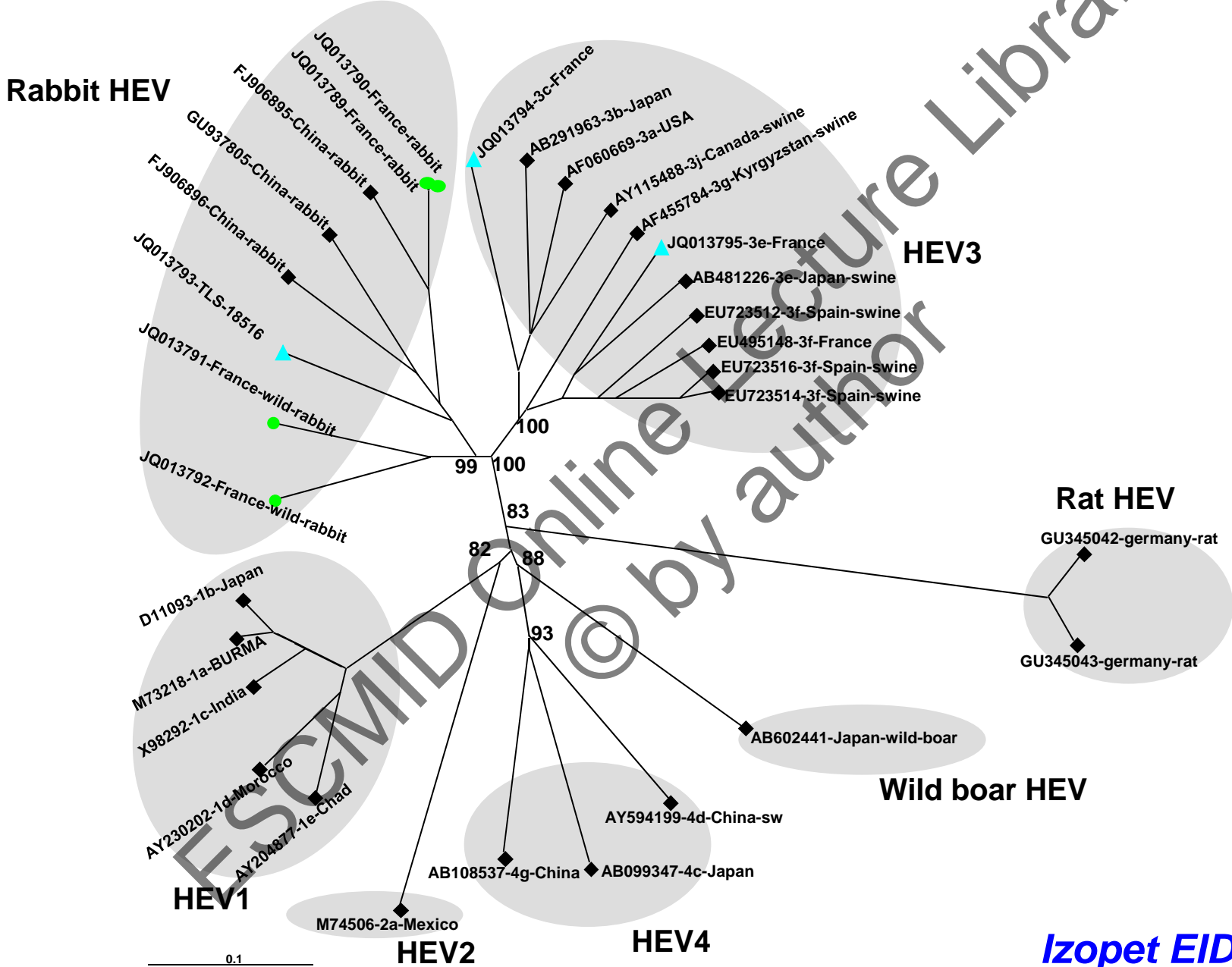
Evidence of HEV reinfection

- ✓ The concentration of anti-HEV antibody that prevents infection has not been defined
 - a vaccine study suggested that 2.5 WHO units/ml could be protective
Shrestha NEJM 07
- ✓ Prospective study of 53 anti-HEV IgG (+) solid-organ transplants
 - no change in anti-HEV IgG concentration after transplantation : 6.6 (0.25-70) IU/ml
 - 2/53 (3.8 %) pts had elevated ALT and a positive HEV RNA within 1 year follow-up : pre-transplant anti-HEV concentration was 2.1 IU/ml and 6.2 IU/ml

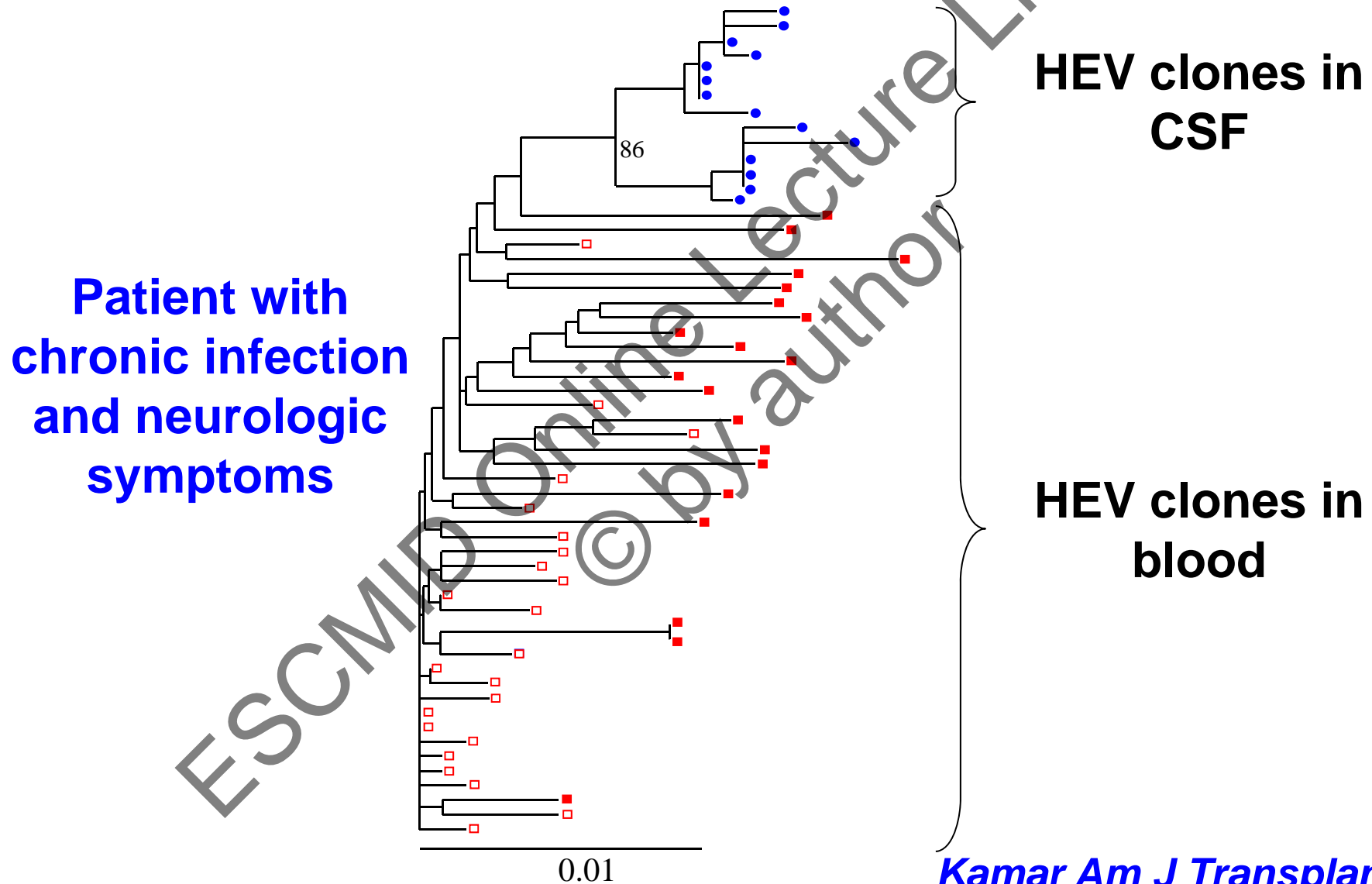
HEV genome sequencing & inter-individual variability

- ✓ Genetic relationship between swine strains circulating in France and human strains
Abravanel EID 09
- ✓ Similar distribution of HEV3 subtypes in swine and humans in France : 3f (74 %), 3c (13 %), 3e (5 %)
Bouquet EID 11
- ✓ Discovery of new HEV genotypes

Full-length HEV sequences



Clonal HEV genome sequencing & intra-individual variability





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EUROPEAN SOCIETY
OF CLINICAL MICROBIOLOGY
AND INFECTIOUS DISEASES

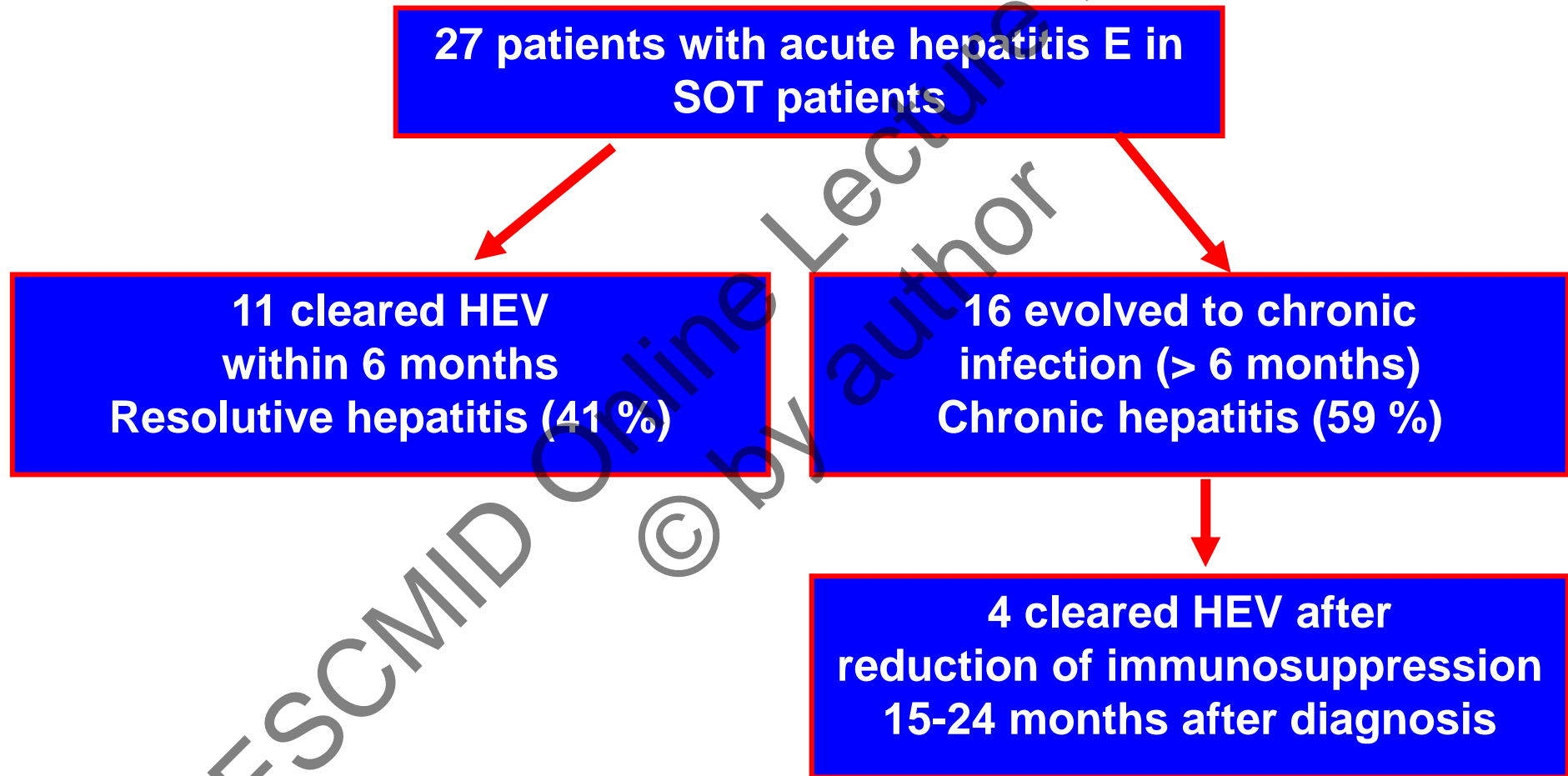
Online Lecture Library

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at request of author**

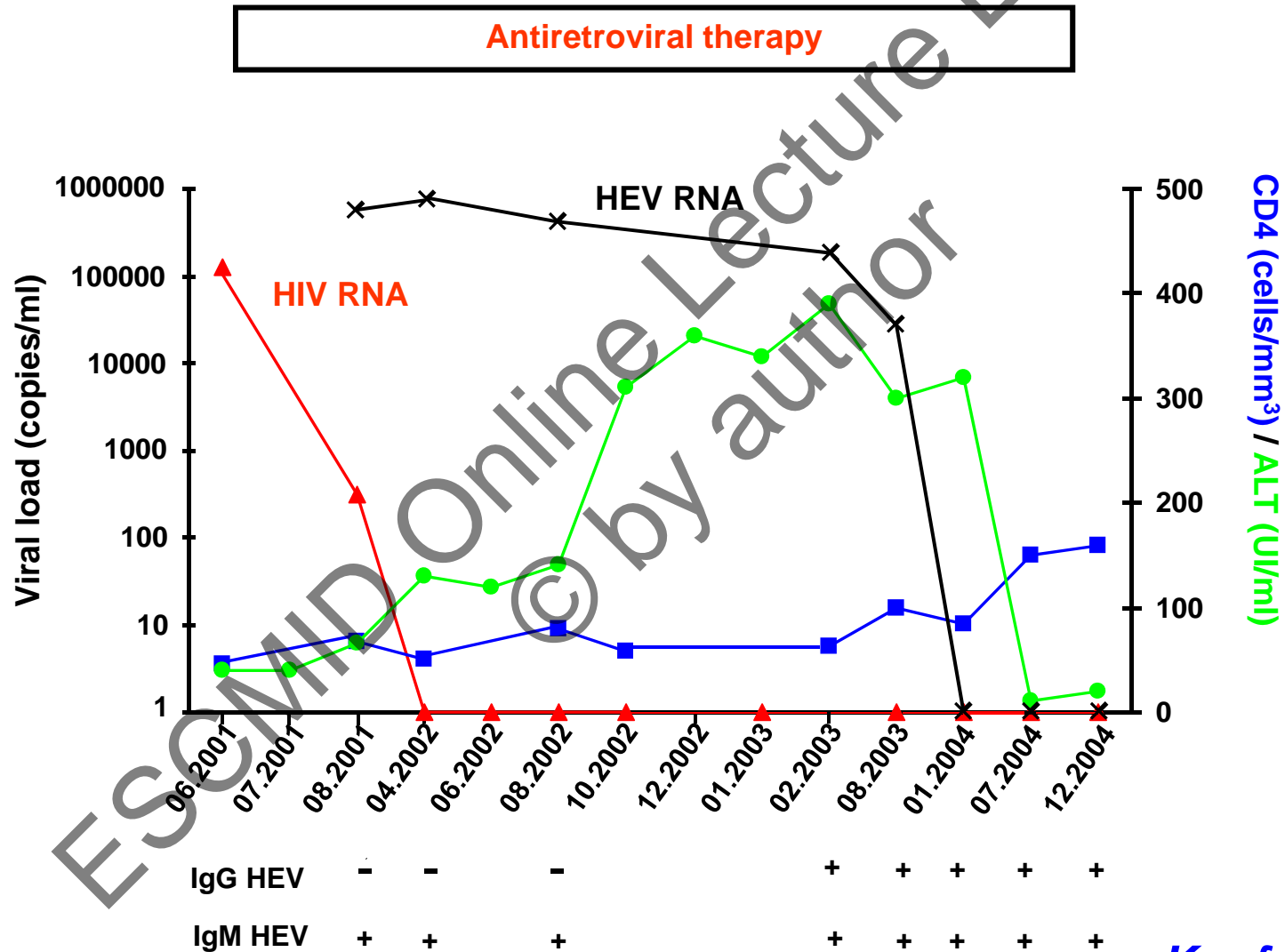
HEV vaccines & capsid protein

- ✓ **GSK candidate vaccine produced in insect cells**
 - 95 % efficacy after 3 doses 0-1-6 (phase II)
Shrestha NEJM 07
- ✓ **Chinese candidate vaccine HEV 239 produced in E coli :**
 - 100 % seroconversion rate after 3 doses 0-1-6 (phase II)
Zhang Vaccine 09
 - 100 % efficacy after 3 doses 0-1-6 (phase III)
Zhu Lancet 10

Reduction of immunosuppressive drugs



HIV-HEV coinfection



Pegylated alpha-interferon for chronic HEV infection

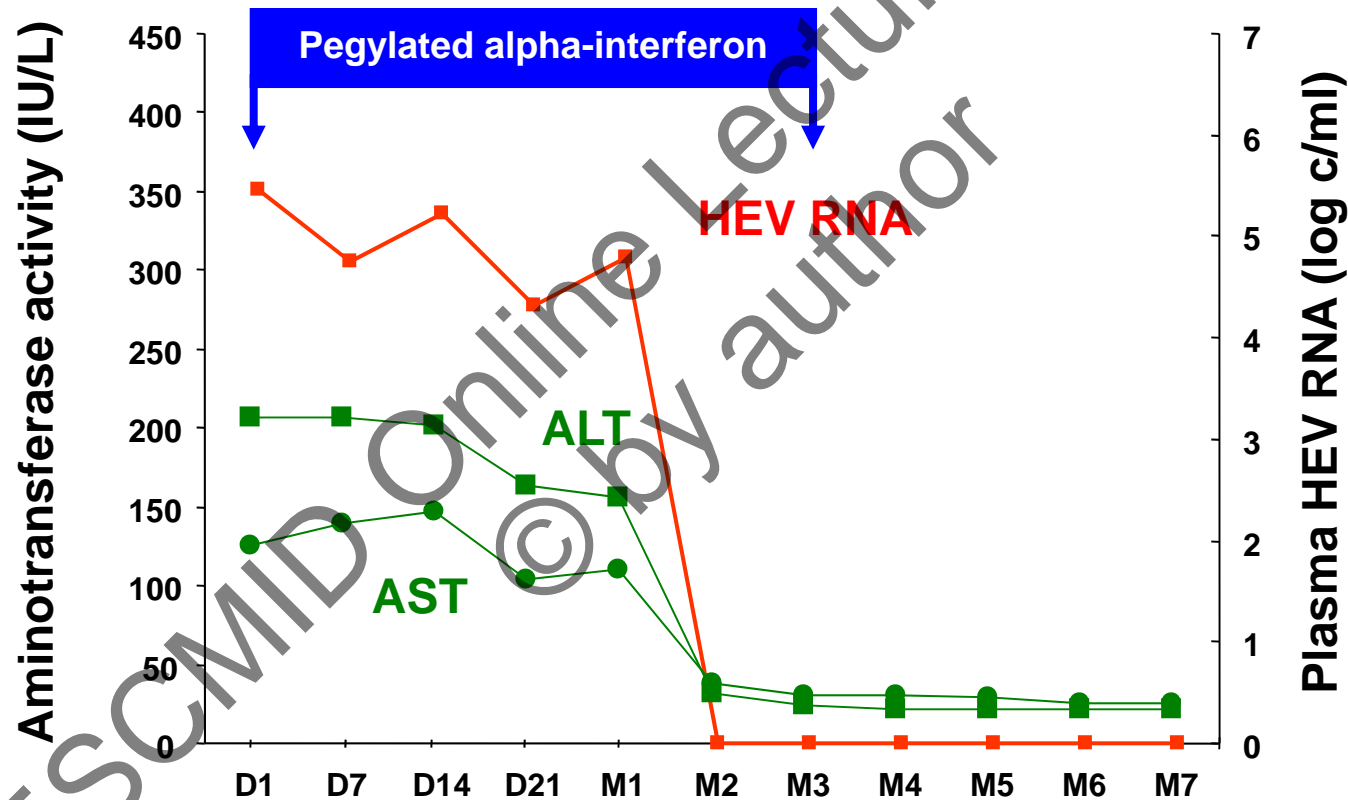
- ✓ **Case series of 3 liver-transplant patients**
 - Peg IFN- α 2a 135 μ g/week for 3 months
 - Sustained virological response in 2/3 patients

Kamar Clin Infect Dis 10
- ✓ **Case series of 2 liver-transplant patients**
 - Peg IFN- α 2b 1.5 μ g/kg/week for 12 months
 - Sustained virological response in 1/2 patients

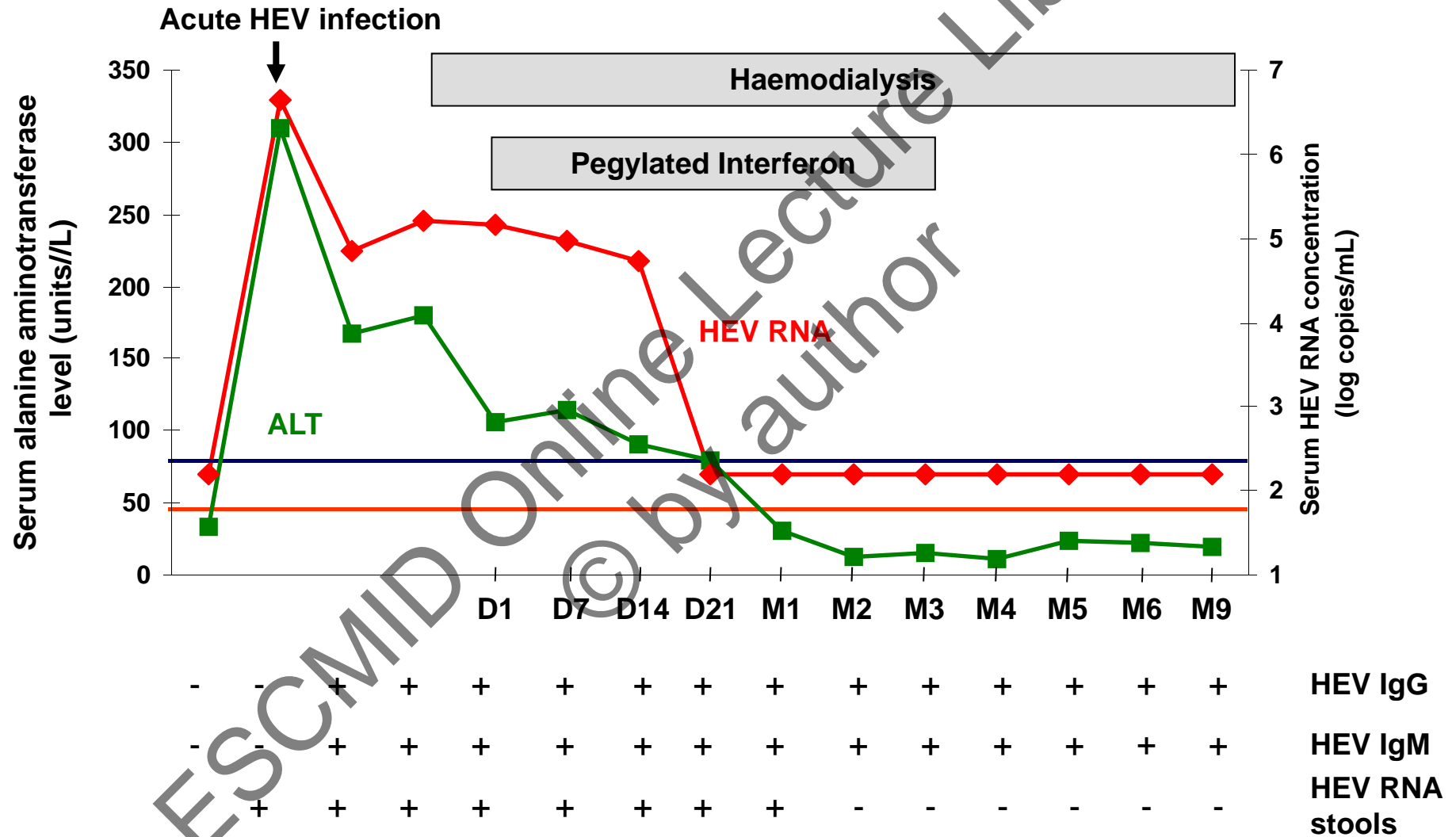
Haagsma Liver Transplant 10

Antiviral effect of Peg-IFN

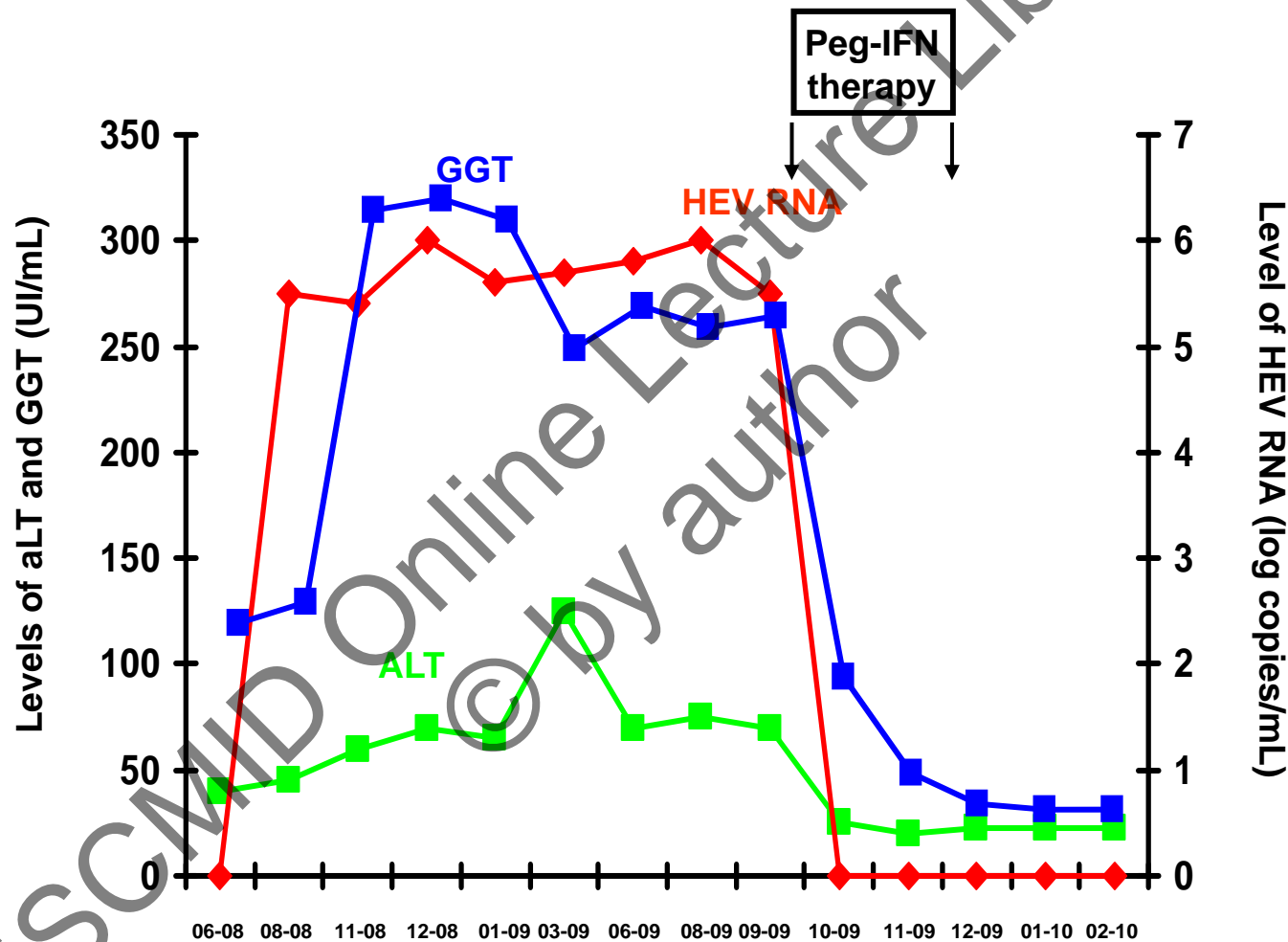
Peg IFN- α 2a 135 μ g/week for 3 months



Peg-IFN in a hemodialysis patient



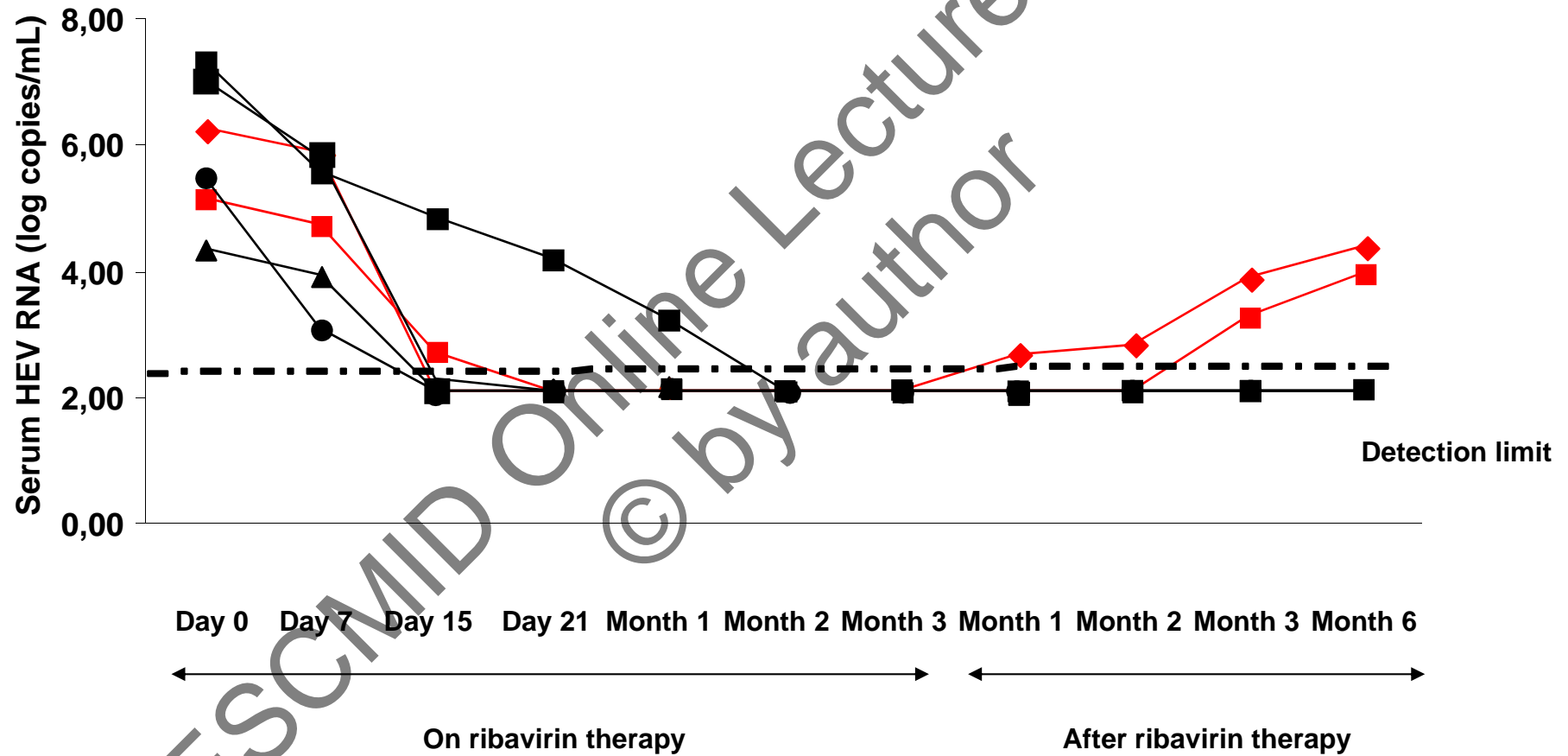
Peg-IFN in a patient with hairy cell leukemia



Stool HEV	+	+	+	+	+	+	+	+	+	-	-	-
HEV IgG	+			+			+	+		+	+	
HEV IgM	+			+			+	+		-	-	

Alric
Ann Intern Med 10

Antiviral effect of ribavirin



Ribavirin treatment

- ✓ Chronic infection when reduction of immunosuppression is not possible

*Mallet Ann Intern Med 10 ; Dalton Ann Intern Med 11 ;
Alric Am J Gastroenterol 11*

- ✓ Acute infection in the context of preexisting chronic liver disease

Peron J Hepatol 11 ; Gerolami JCV 11

Summary

- ✓ **Better understanding of HEV infection thanks to serologic & molecular techniques**
 - **autochthonous zoonotic transmission**
 - **chronic infections in immunocompromised patients**
 - **hepatic and extra-hepatic manifestations**
- ✓ **Pegylated interferon and ribavirin can eradicate HEV**
- ✓ **Promising results with HEV vaccines**

Acknowledgments

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