LIVER PATHOLOGY (1)

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Liver Pathology (1)

- Structure of the liver
- General reactions of the liver
- Diagnosis of liver diseases
- Congenital liver diseases, enzymopathies
- Infectious liver diseases
  - Viral hepatitis
- Chronic hepatitis
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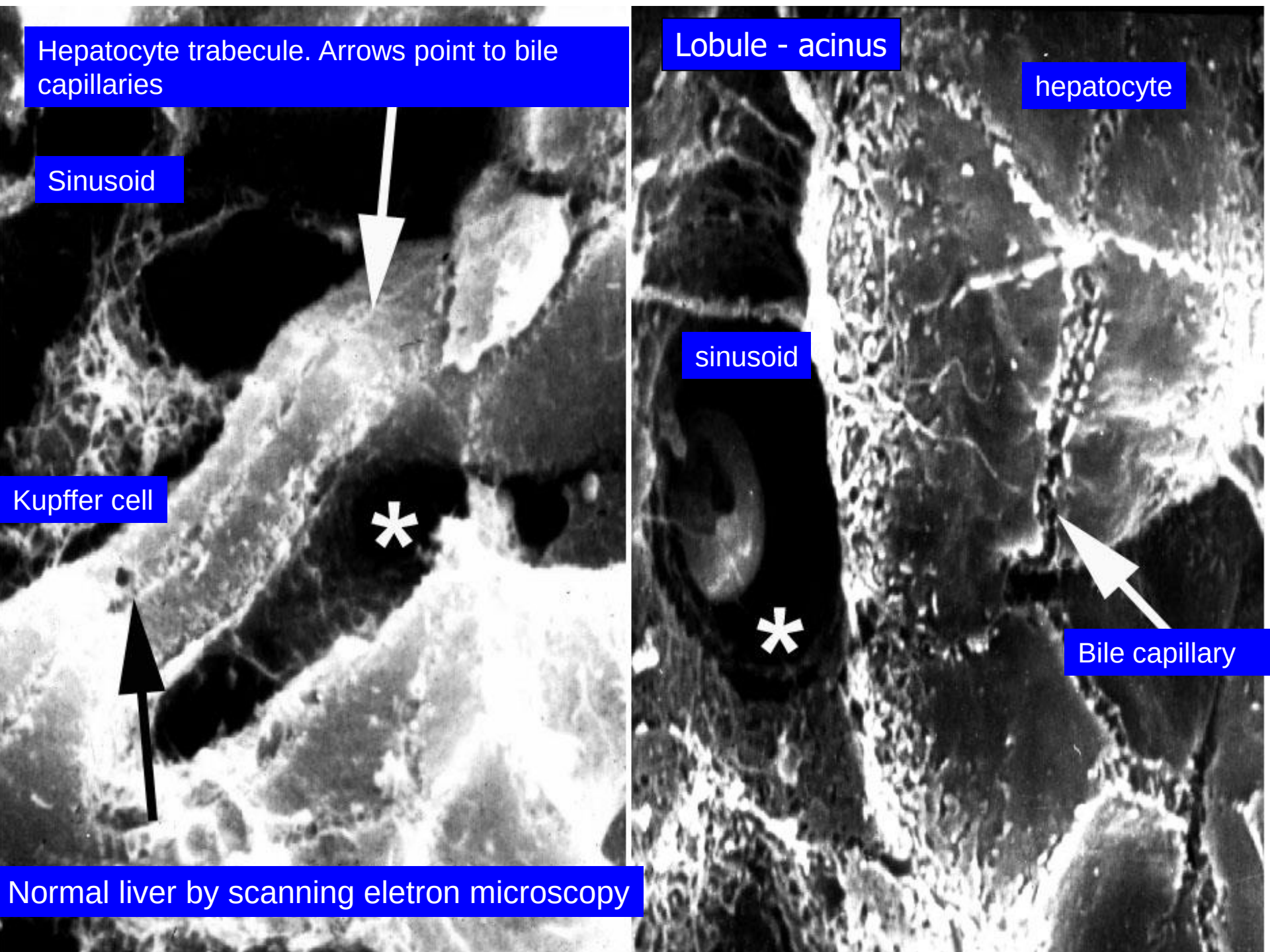
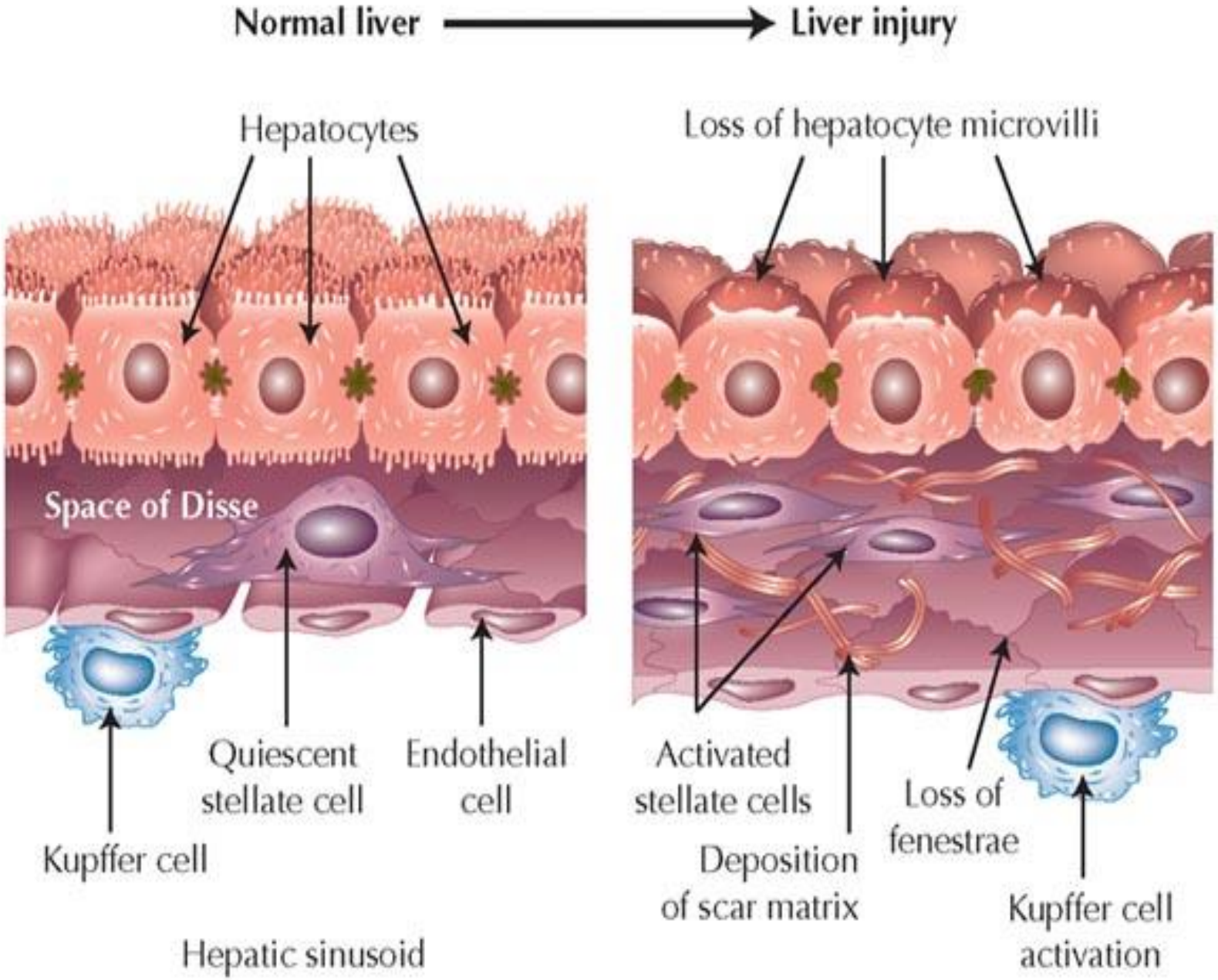
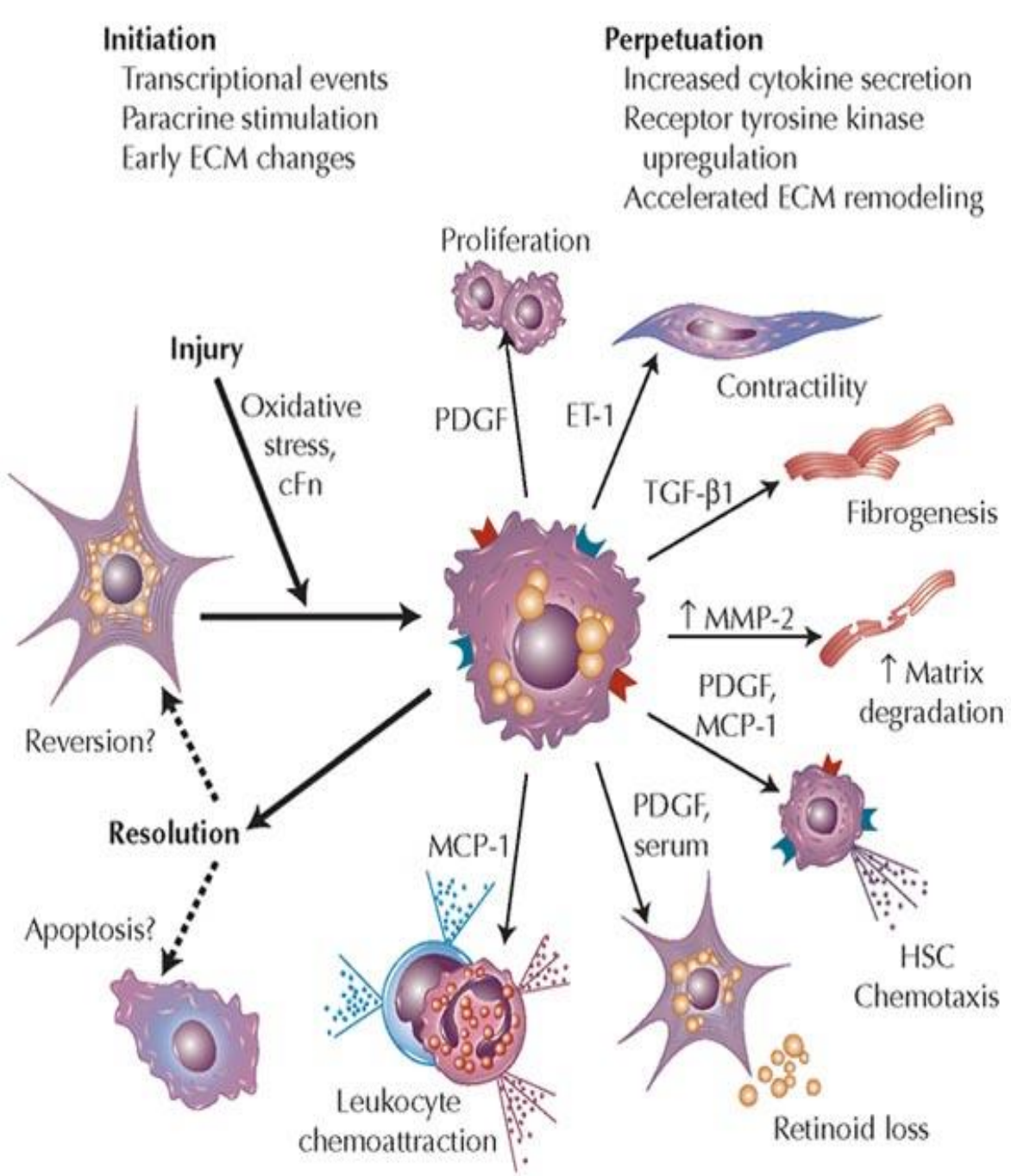


Normal liver by scanning eletron microscopy

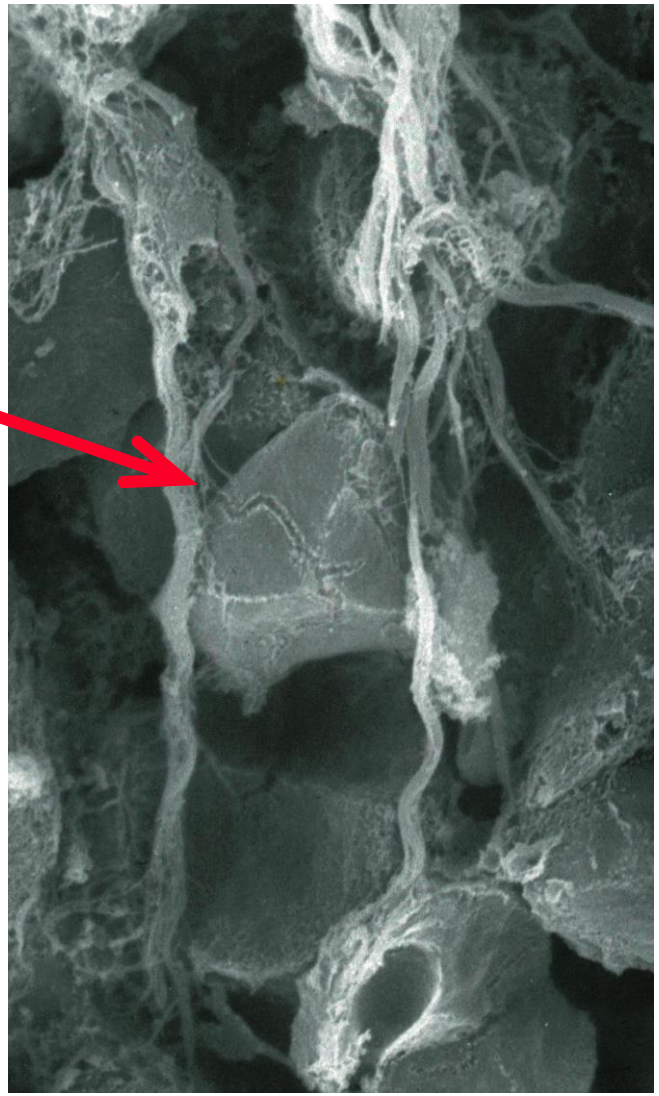

Normal liver
Liver injury
Hepatocytes
Loss of hepatocyte microvilli
Space of Disse
Quiescent stellate cell
Endothelial cell
Kupffer cell
Activated stellate cells
Deposition of scar matrix
Loss of fenestrae
Kupffer cell activation
Hepatic sinusoid

„Stellate cell”



Types of liver cells (**)

- **Parenchymal cells**
  - Hepatocytes (arrow)
  - Biliary cells
- **Non parenchymal cells**
  - Endothelial
  - Kupffer cells
  - Stellate (Ito, fat storing)

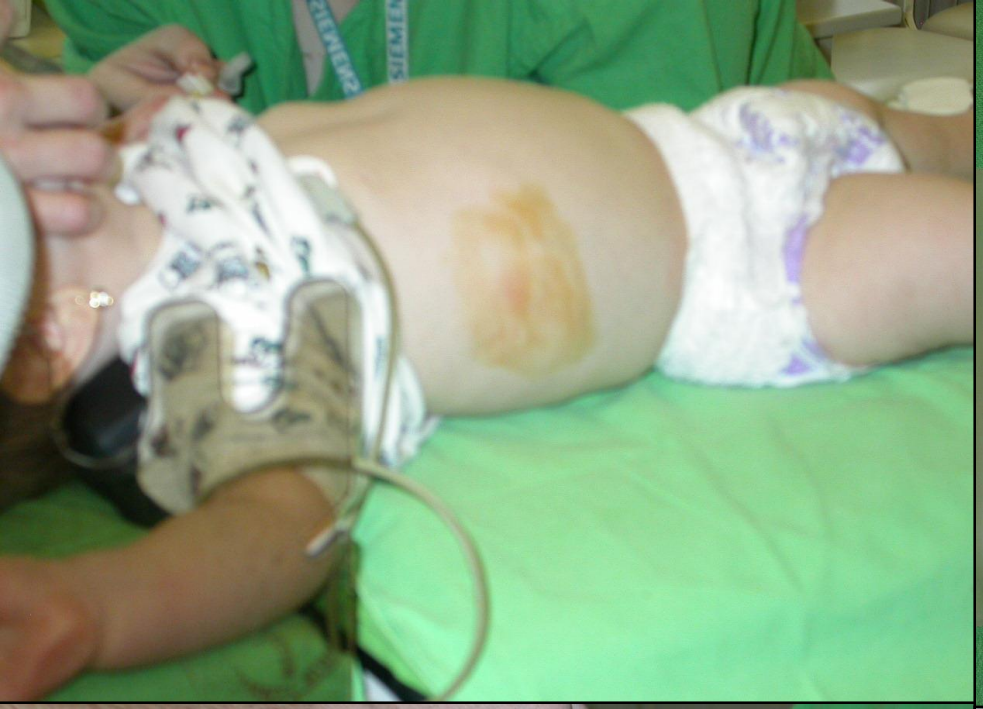


Basic reaction types of the liver (**)

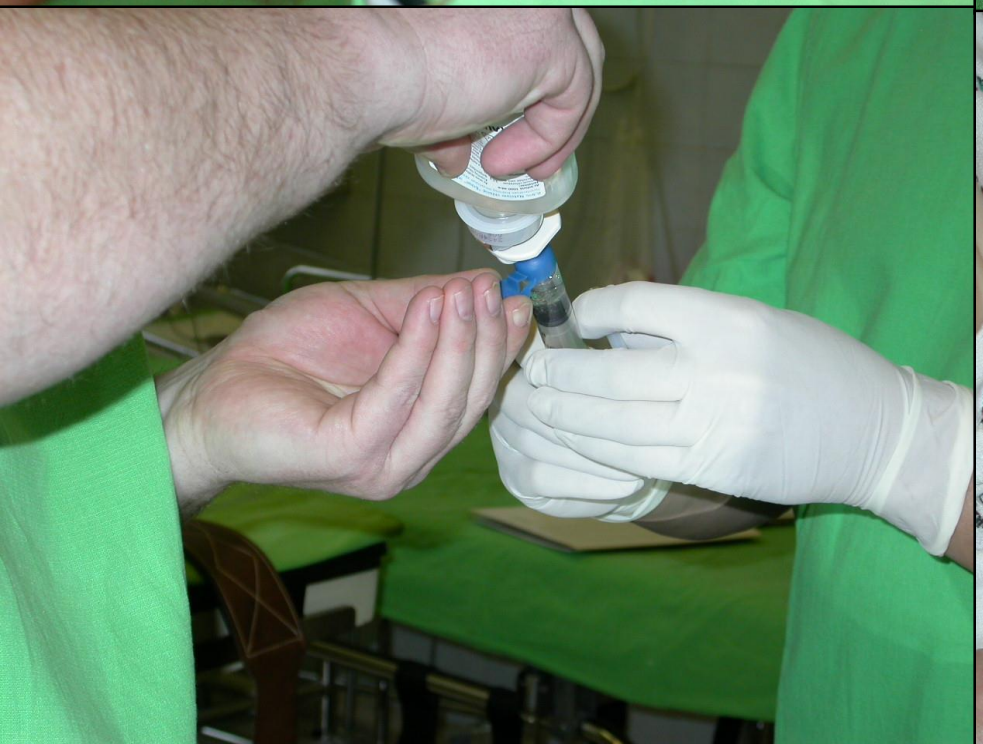
- Reversible changes
  - adaptive
  - degenerations
  - Intracellular accumulation
- Irreversible changes
  - Cell death: necrosis, apoptosis
- Regeneration/proliferation
- Inflammation
- Neoplastic transformation

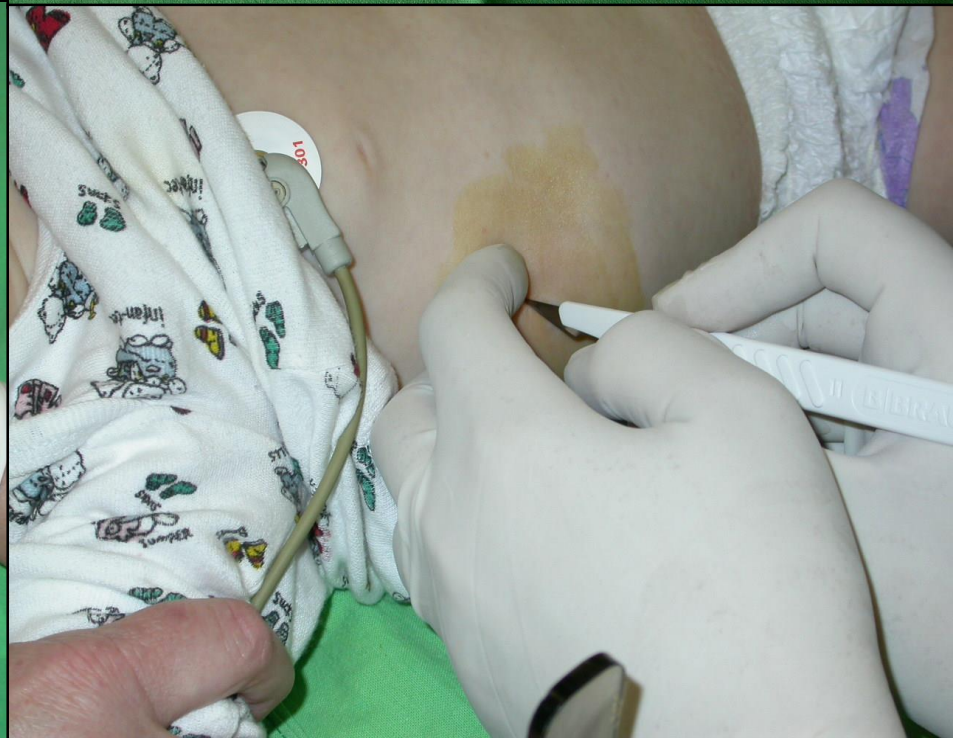
Methods in diagnostics of liver diseases (**)

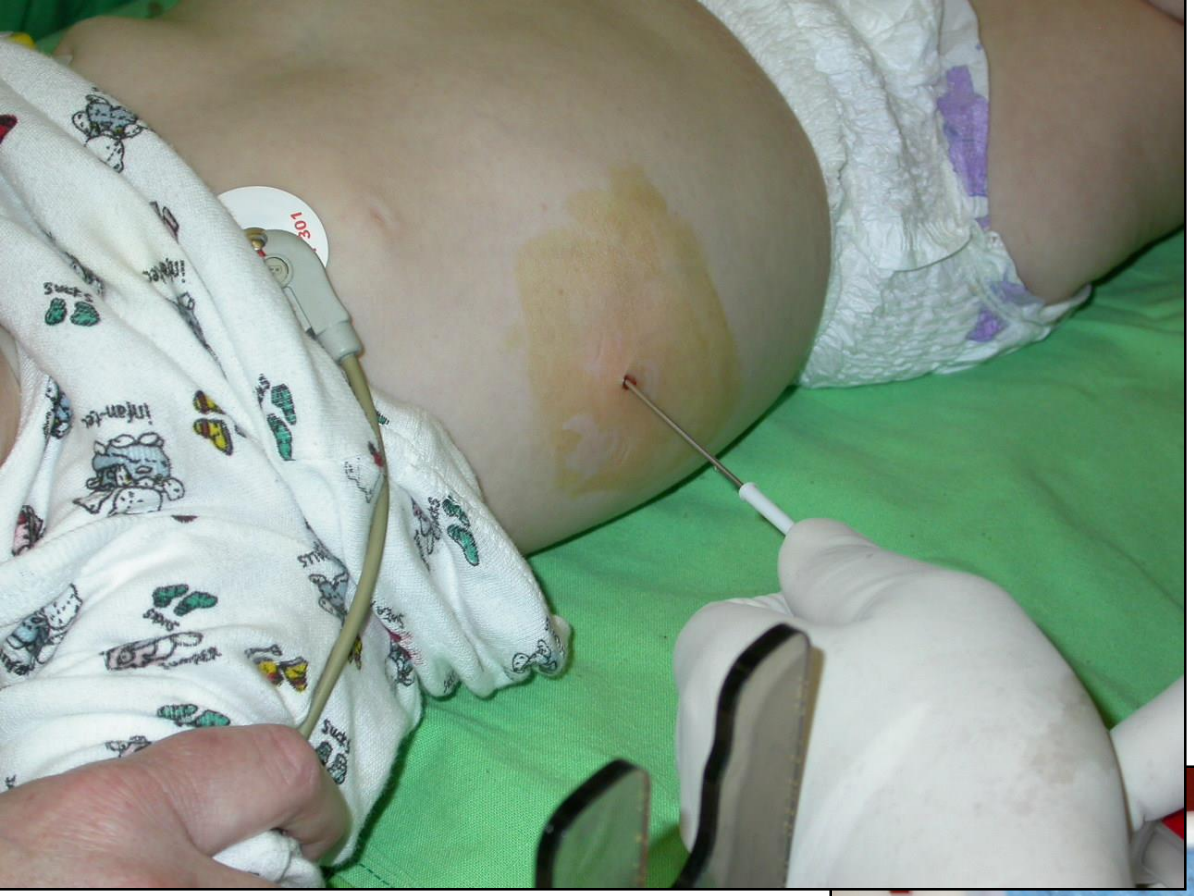
- Needle biopsy
  - Histology
  - Sections: H&E, PAS, ePAS, Berlini blue, connective tissue stains, Shikata - orcein, etc, immunohistochemistry
- Fine needle aspiration biopsy
  - cytology
- „Surgical" („wedge") biopsy



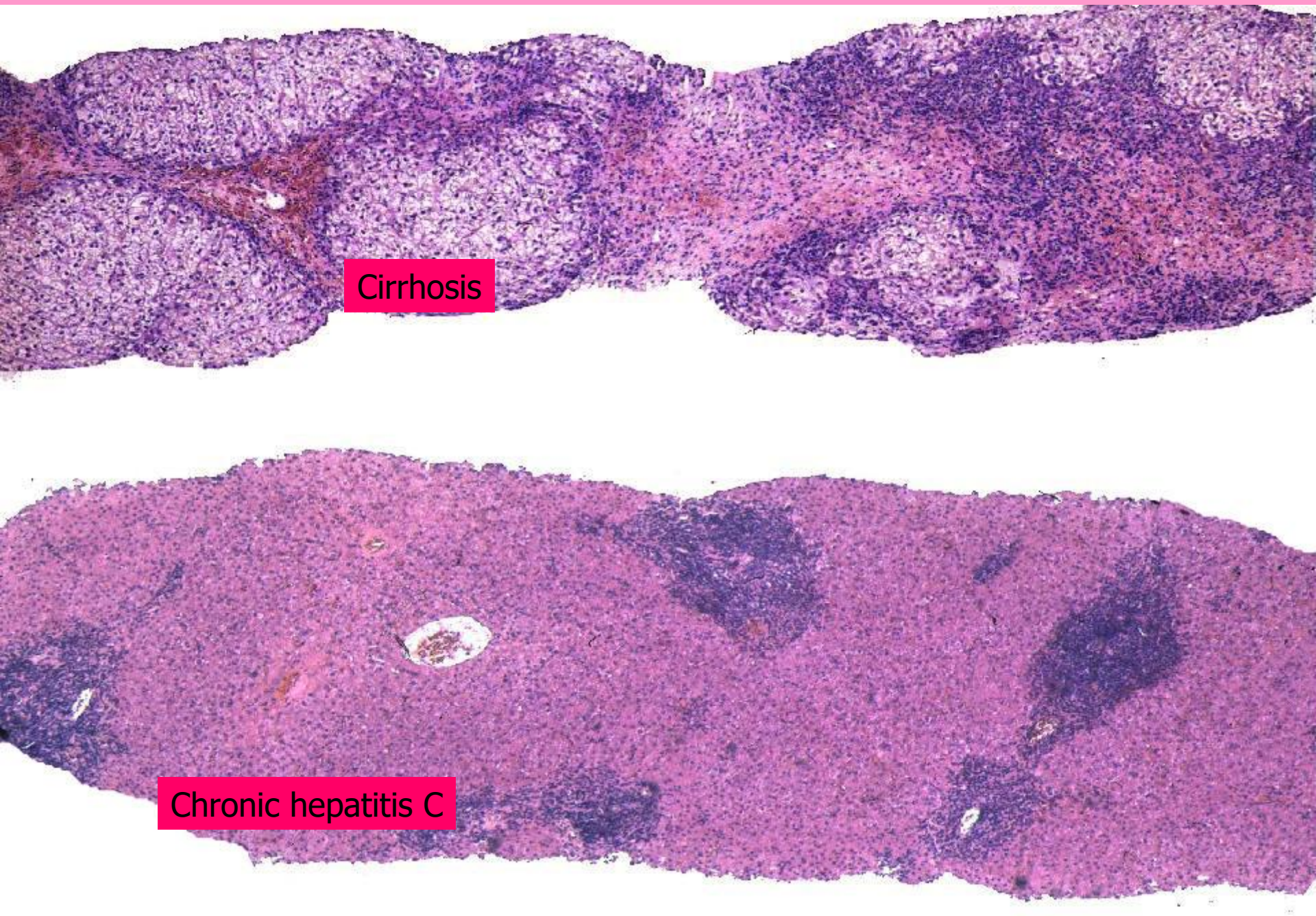

Hepafix®
B|BRAUN







Needle biopsy samples. HE staining.



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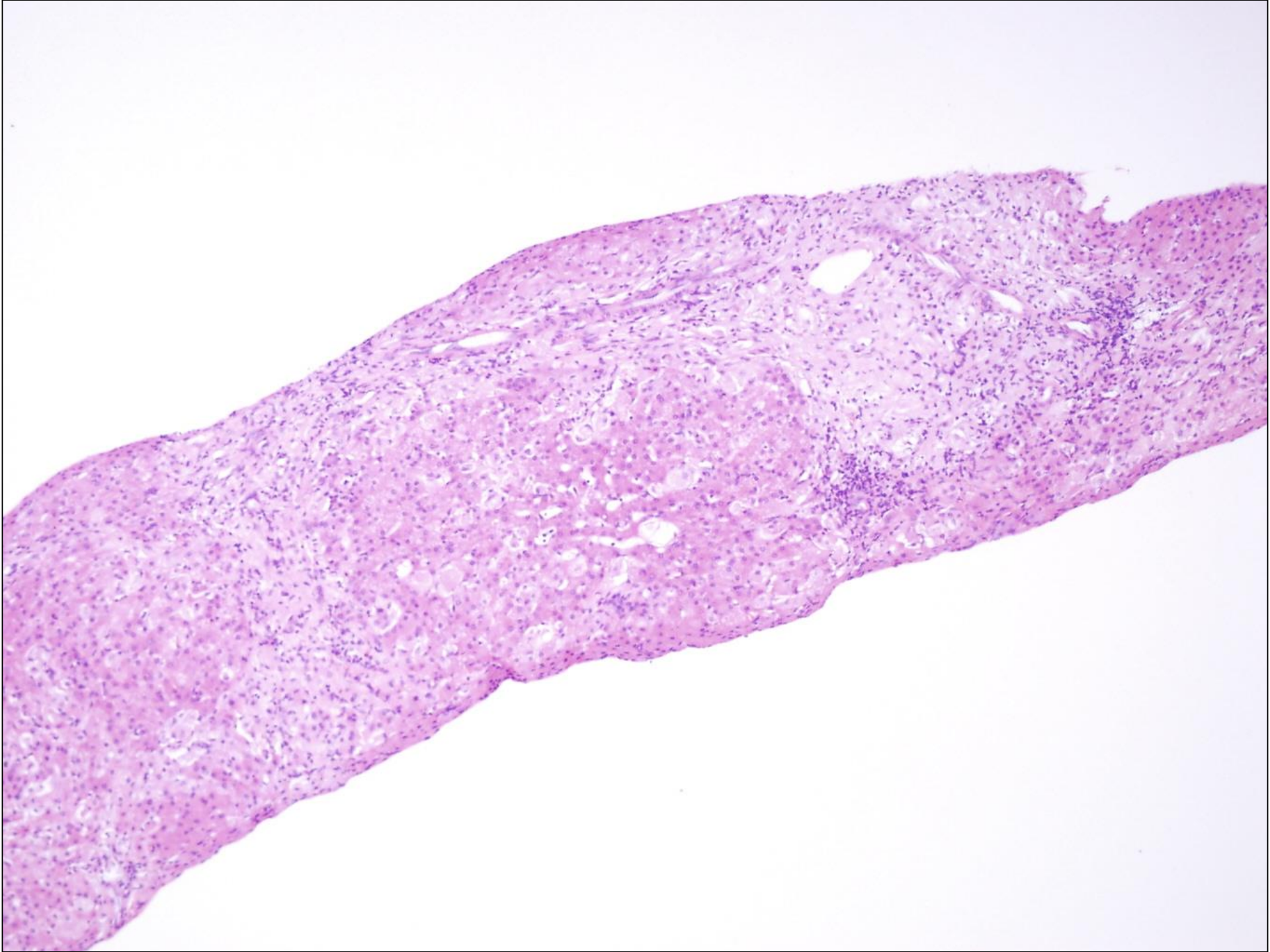
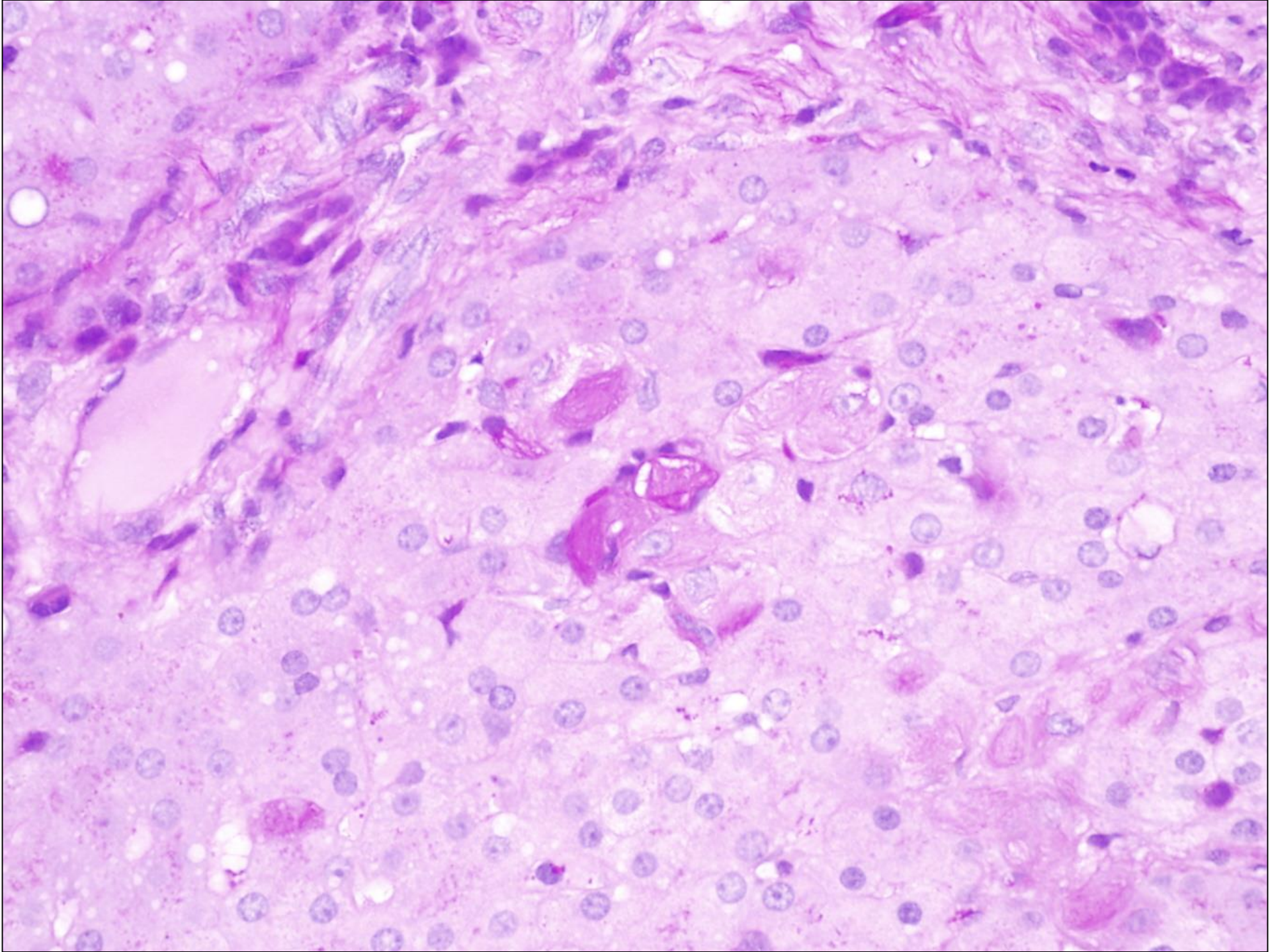
Congenital liver diseases, enzymopathies (1) (**)

- Haemochromatosis (Fe)
  - „bronzdiabetes”: liver (cirrhosis), pancreas (fibrosis, atrophy), skin discoloration
- Wilson disease (Cu) (AR)
  - Liver (CAH, cirrhosis), CNS (basal nuclei), eyes (Kaiser-Fleisher ring)
- Alfa-1-antitrypsin deficiency (AR)
  - Serum AAT low, liver (dPAS+ globulusok, cirrh), lung (emphysema), CAVE smoking!!! PiMM (normal), Z,S altered
- Tyrosinaemia (AR)
  - HCC!
- Glycogenosis (AR)
  - Liver is involved in types I., (Gierke) III., IV., VI (hepatomegaly, steatosis, fibrosis, cirrhosis, adenomatosis, HCC)
- Galactosaemia
  - Cataracta, hepatomegaly, cirrhosis. Screenings!!! Diet!
- Gaucher disease (glykozil-ceramid-lipidosis)
  - Most common enzymopathy in the liver, genetic heterogeneity,
  - Gaucher-cells (!!!).
  - Types I.:young, liver II.:infants, children, cerebral alterations III.: CNS and liver alterations, young adults

Case
3 yr old male (10695/04)

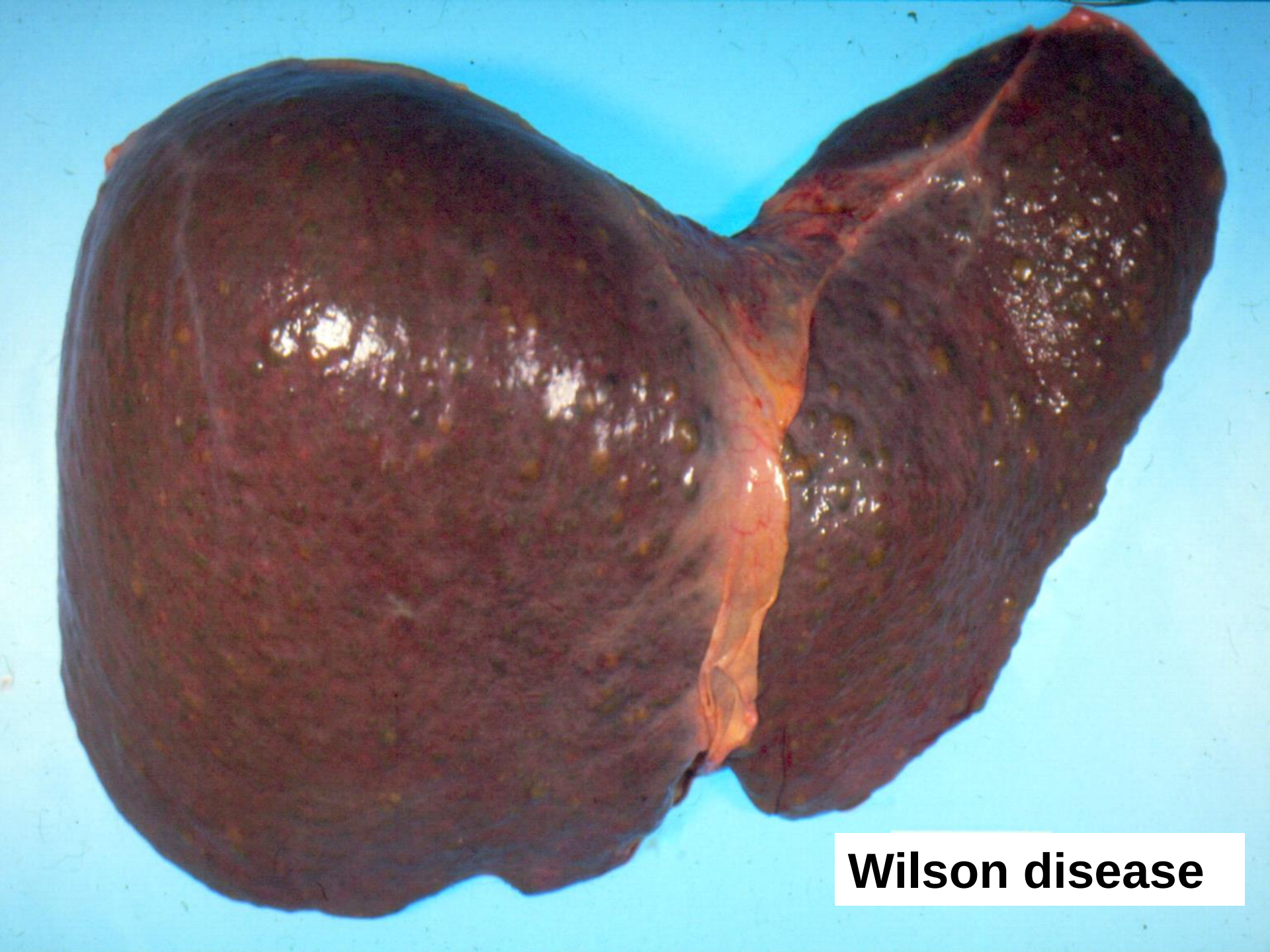
- Hepatosplenomegaly
- Virus serology: negatív
- Clinical question: hematologic disorder?





Diagnosis

- Gaucher disease


Wilson disease

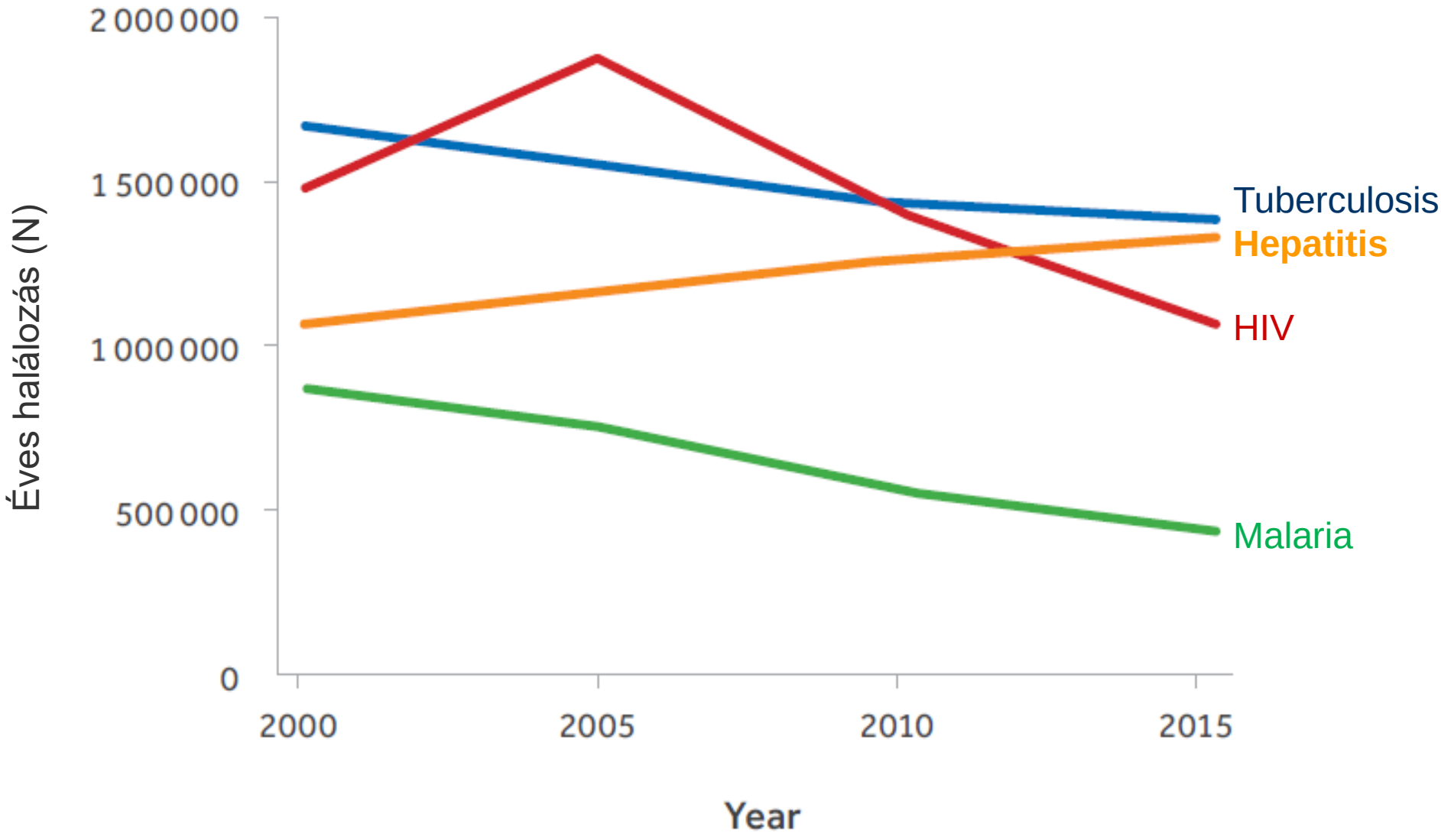
Congenital liver diseases, enzymopathies (2) (**)

- Porphyrias
  - primary (inherited), secondary (liver disease, diabetes etc)
  - Acut intermittent porphyria (long asymptomatic period, manifestation for external effects, no skin alteration, GI and psychotic symptomes (!!!), endocrin alterations (hyponatremia!), hist: UV fluorescence. Urine, stool!
  - Porphyria cutanea tarda (increased UV sensibility, skin alterations, liver: steatosis, porf.precurzor, UV fluorescence)

Congenital liver diseases, enzymopathies (3) (**)

- Herediter hyperbilirubinemias
  - Non-conjugated
    - Crigler-Najjar-syndrome
      - Type I.: fatal (AR) II.: less severe (AD)
    - **Gilbert-syndrome** (AD) (fluctuating icterus,
      - 3-7 %, „normal liver” by histology
  - Conjugated
    - Dubin-Johnson-syndrome (AR) (intermitted jaundice, „black” liver, large pigment granules)
    - Rotor-syndrome (AR) (mild icterus, increased number of lysosomes, „normal liver”)

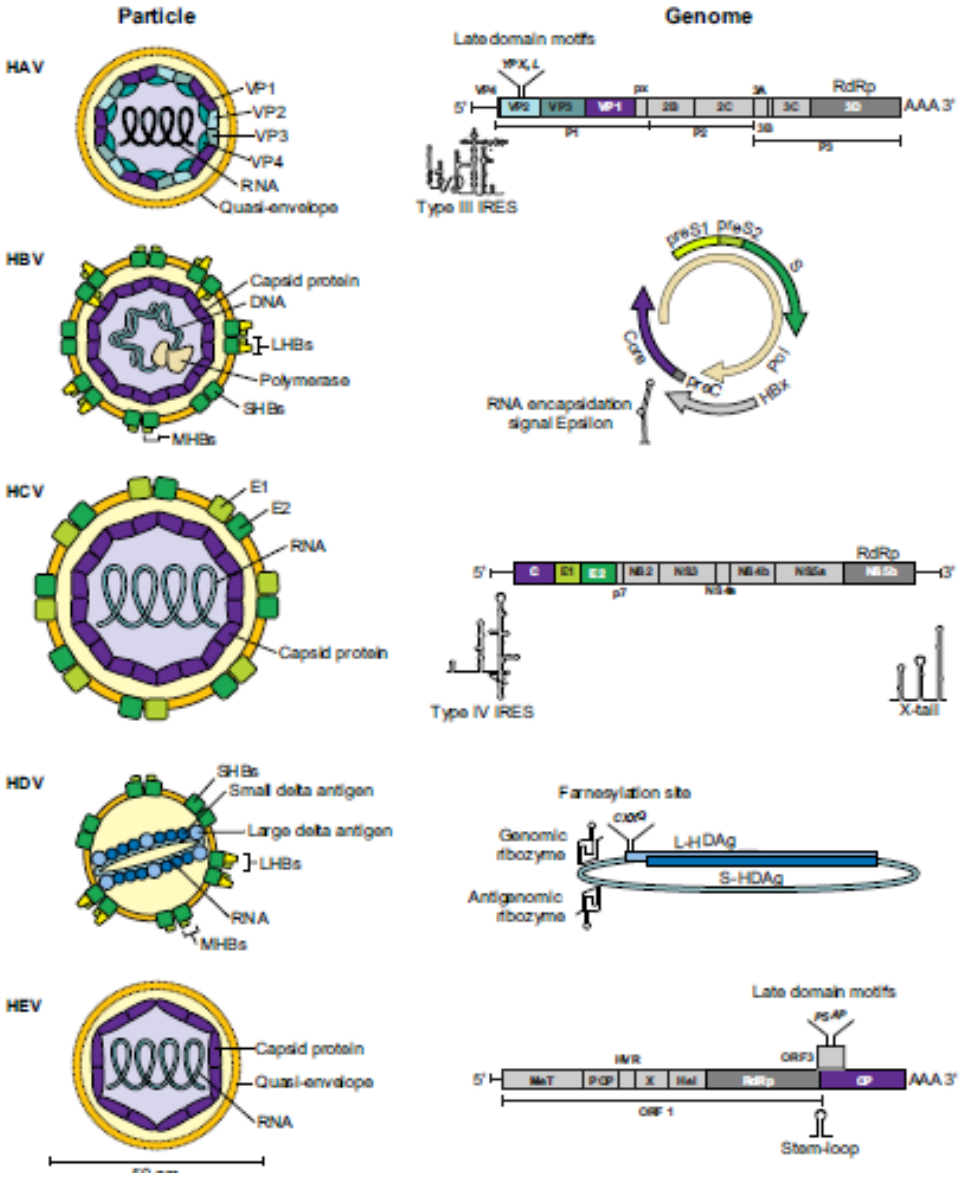
Viral hepatitis-associated mortality shows increasing tendency



http://apps.who.int/iris/bitstream/10665/255016/1/9789241565455-eng.pdf?ua=1

Hepatotropic viruses (***)

	HAV	HBV	HCV	HDV	HEV	
Family	Picorna	Hepadna	Flavi Hepaci	Satellit	Hepe	
Genome	ssRNA	dsDNA	ssRNA	ssRNA	ssRNA	
Virion	27 nm	42 nm	55-65 nm	36 nm	32-34 nm	
Chronicity	-	+	+	+	-/+	

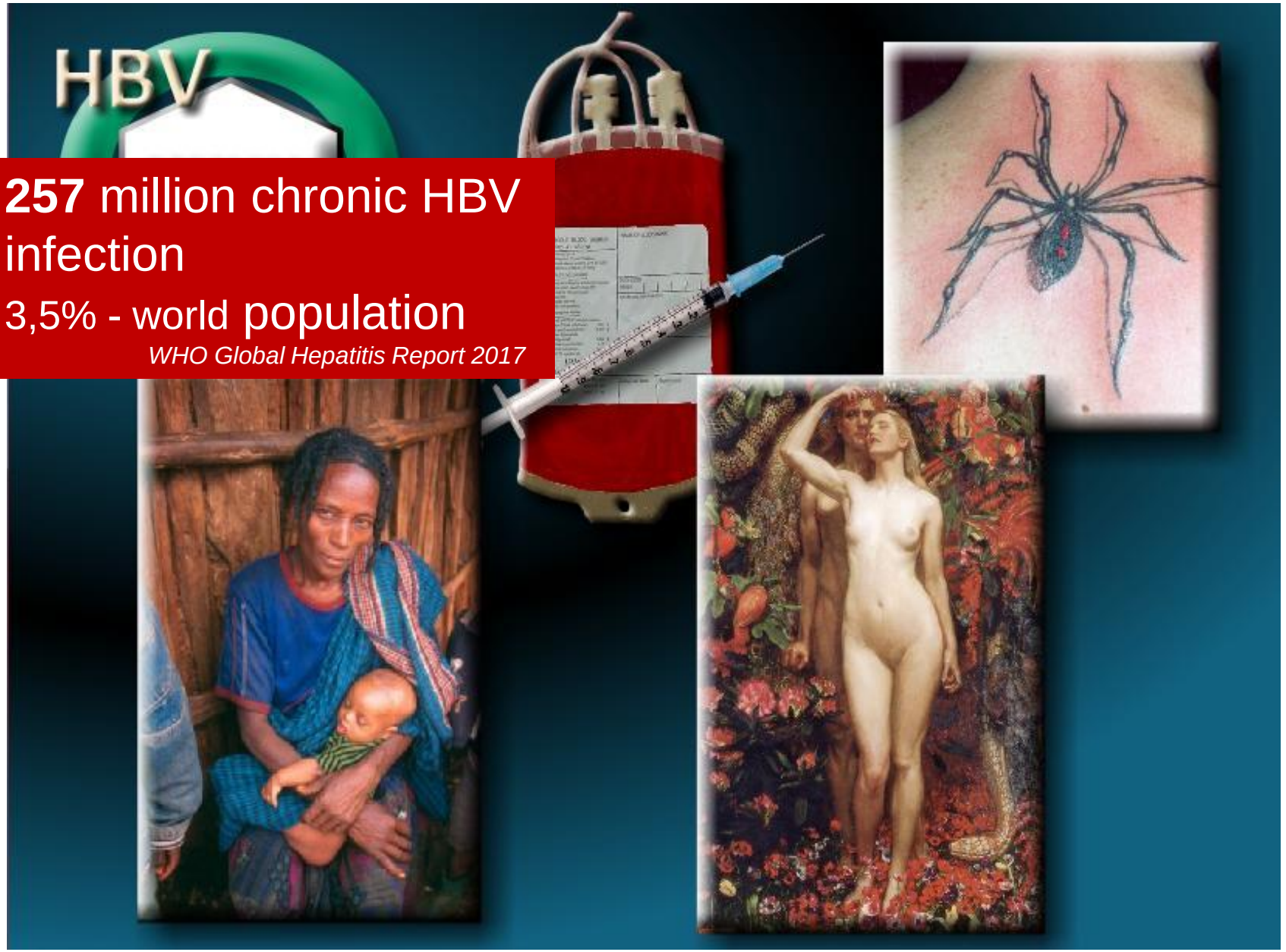

Particle
Genome
HAV
VP1
VP2
VP3
VP4
RNA
Quasi-envelope
Late domain motifs
YPXnL
VP4
VP2
VP3
VP1
2A
2B
2C
3A
3B
3C
3D
RdRp
AAA 3'
P1
P2
P3
Type III IRES
HBV
Capsid protein
DNA
LHBs
Polymerase
SHBs
MHBs
preS1
preS2
S
Core
preC
Pol
HBx
RNA encapsidation signal Epsilon
HCV
E1
E2
RNA
Capsid protein
RdRp
C
E1
E2
NS2
NS3
NS4b
NS5a
NS5b
p7
Type IV IRES
X-tail
HDV
SHBs
Small delta antigen
Large delta antigen
LHBs
RNA
MHBs
Farnesylation site
Genomic ribozyme
L-HDAg
S-HDAg
Antigenomic ribozyme
HEV
Capsid protein
Quasi-envelope
RNA
Late domain motifs
PSAP
ORF3
HVR
MeT
PCP
X
Hel
RdRp
CP
AAA 3'
ORF 1
Stem-loop

Chronic infection with viral hepatitis

- Global prevalence
  - **HBV: 257 million (3,5%)**
  - **HCV: 71 million (1%)**
- Geographical differences
  - HBV: <1 – 20%
  - HCV: <1 – 10%
- Chronic inflammation
  - HBV: appr. 10%
  - HCV: appr. 70-80 %

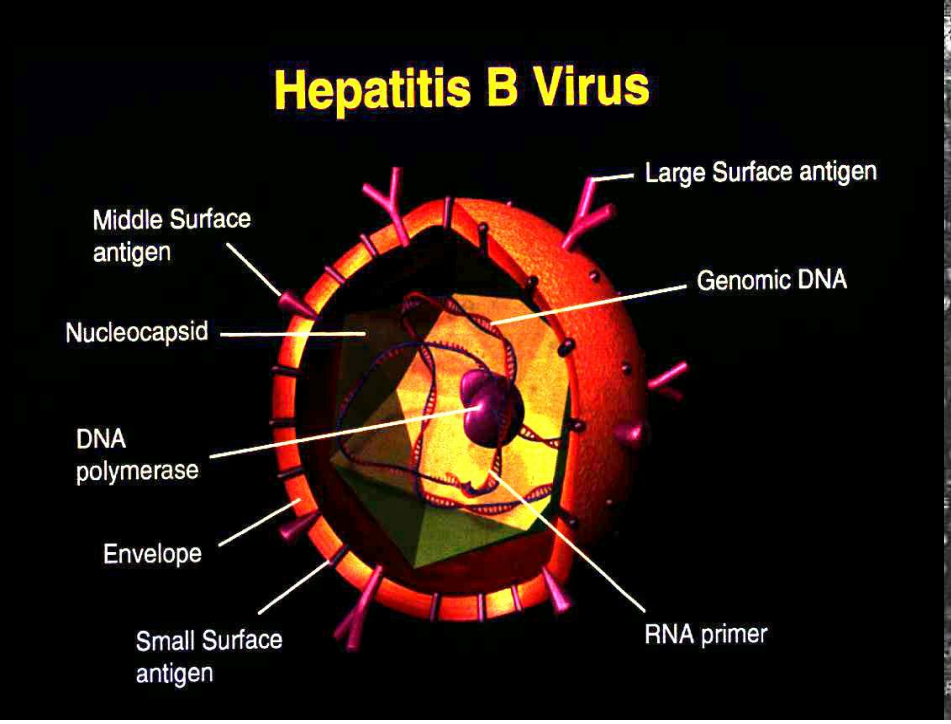
Other viruses causing hepatitis

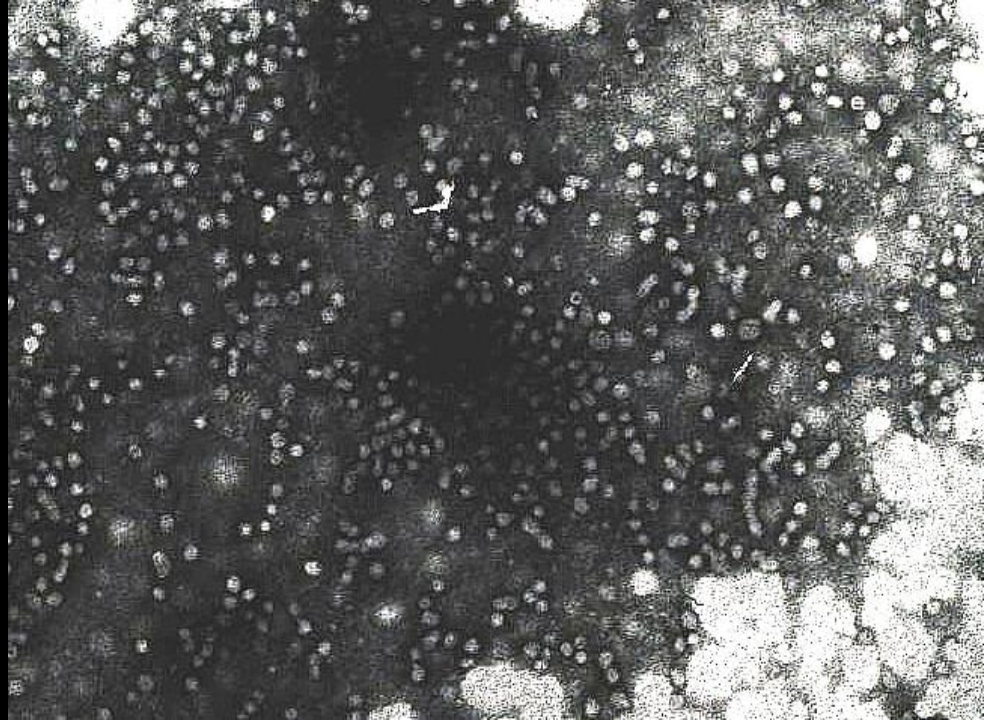
- CMV
- EBV
- HHV-6, -7
- Human parvovirus B19 (small, ssDNA virus)
- TTV (unenvelopesd xDNA virus)

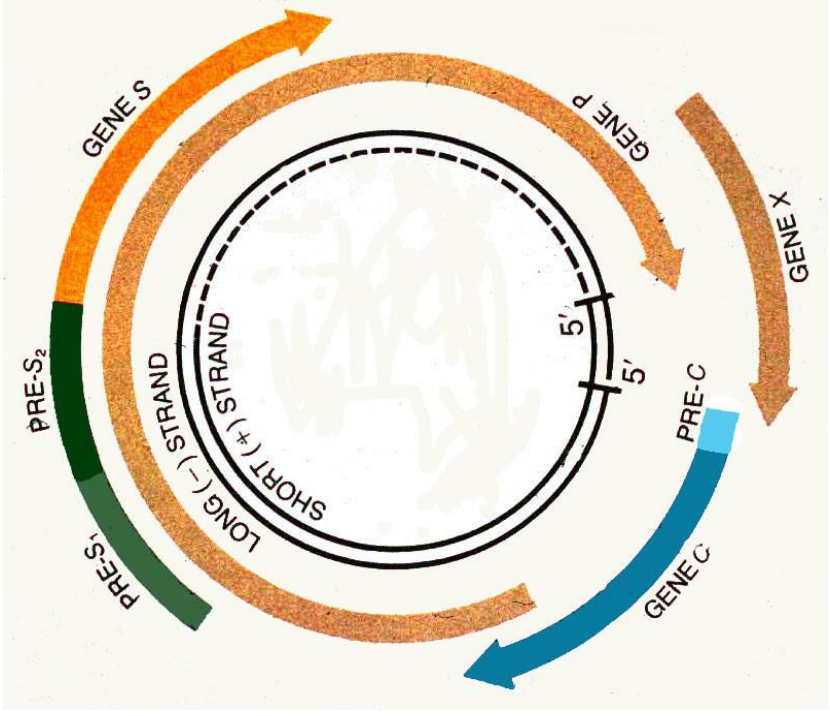

HBV
- **257** million chronic HBV infection
- 3,5% - world population
WHO Global Hepatitis Report 2017

- **257** million
- 0,1%-6,2%
  - Far East: 6,2%
  - Africa: 6,1%
  - Eastern Mediterranian: 3,3%
  - South-East Asia: 2,0%
  - Europe: 1,6%
  - America: 0,7%

WHO Global Hepatitis Report 2017







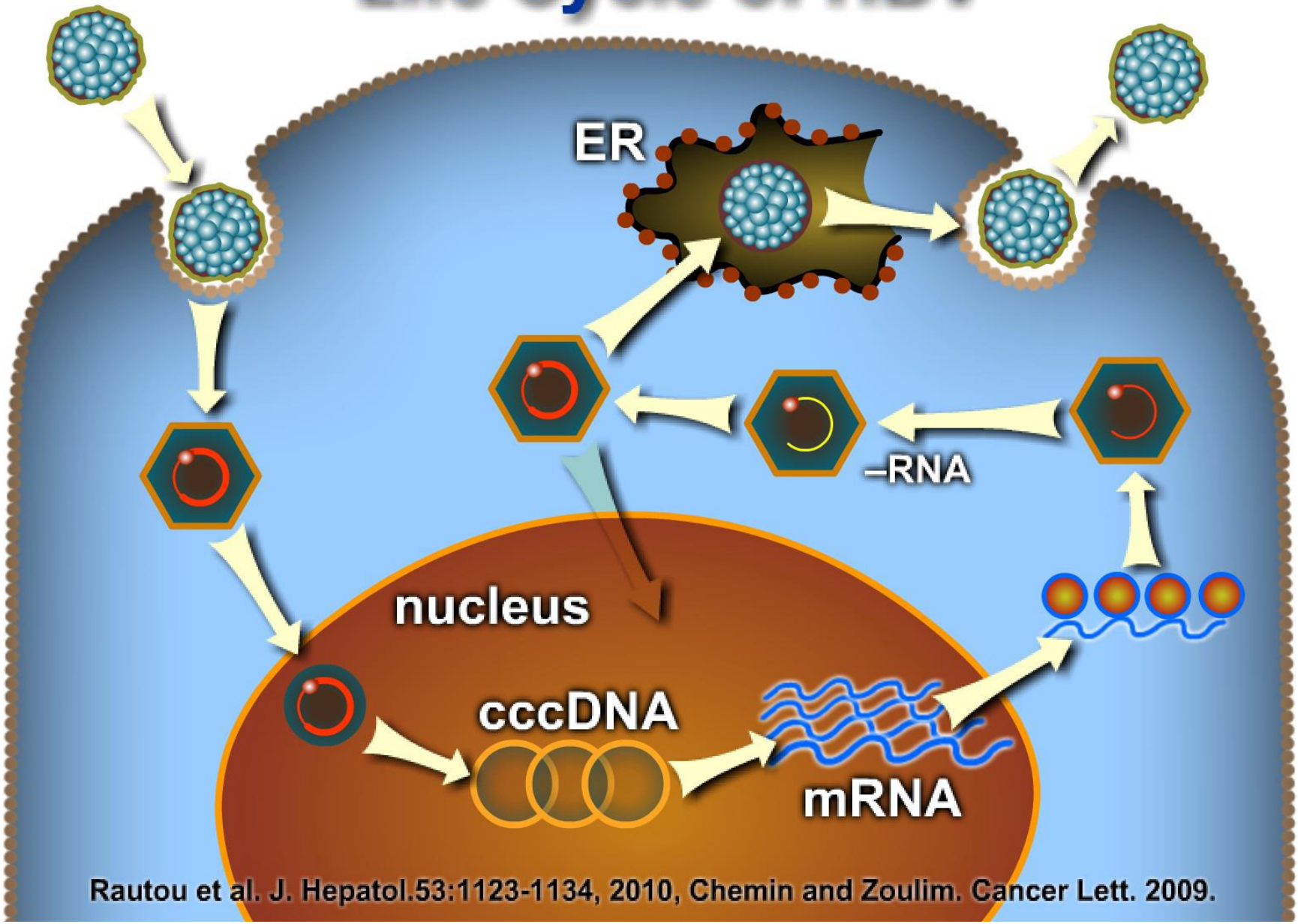
Hepadnaviridae

dsDNA (**)

RT

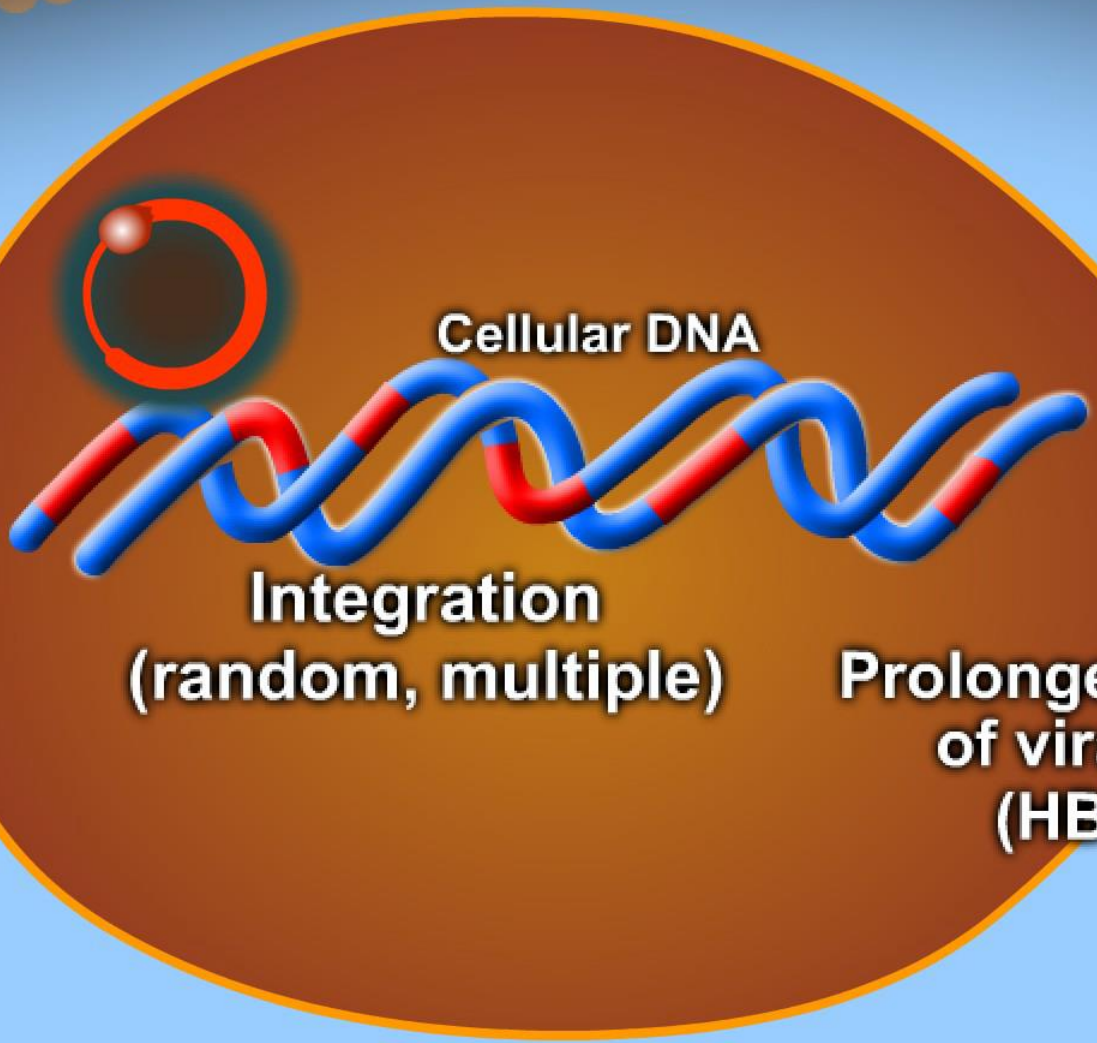
Core, surface Ag

Life Cycle of HBV

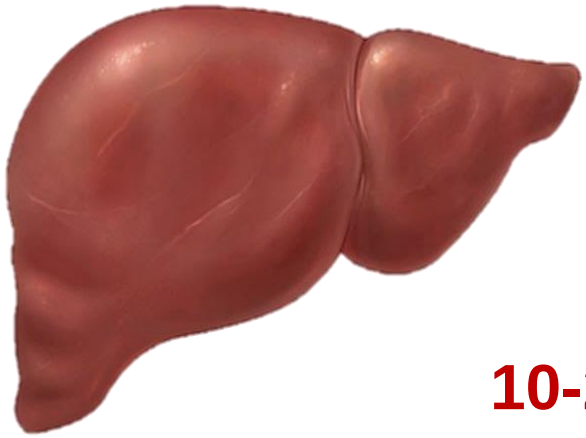


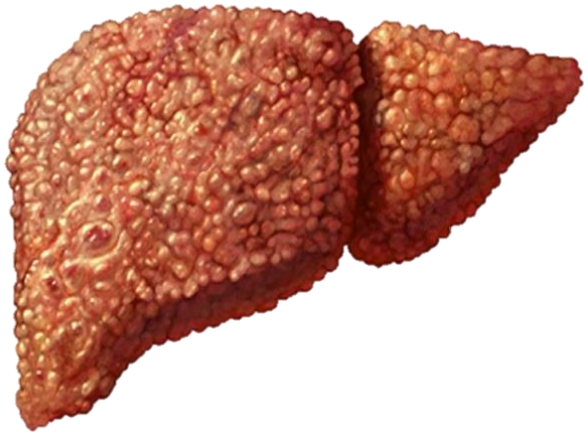
Rautou et al. J. Hepatol.53:1123-1134, 2010, Chemin and Zoulim. Cancer Lett. 2009.

Insertion of HBV Genome in Cellular Genes



Viral gene persistence





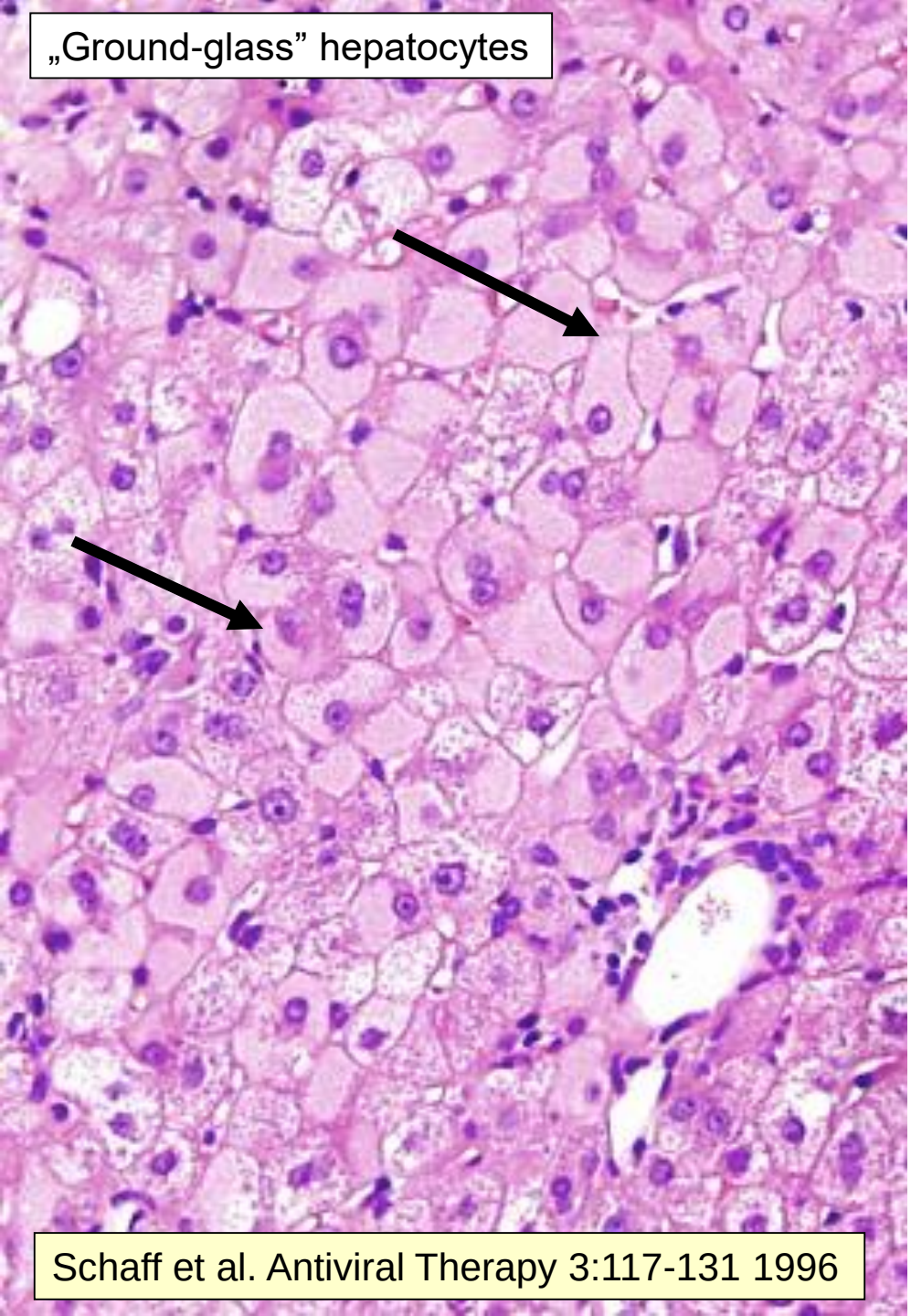
10-20 years

HBV

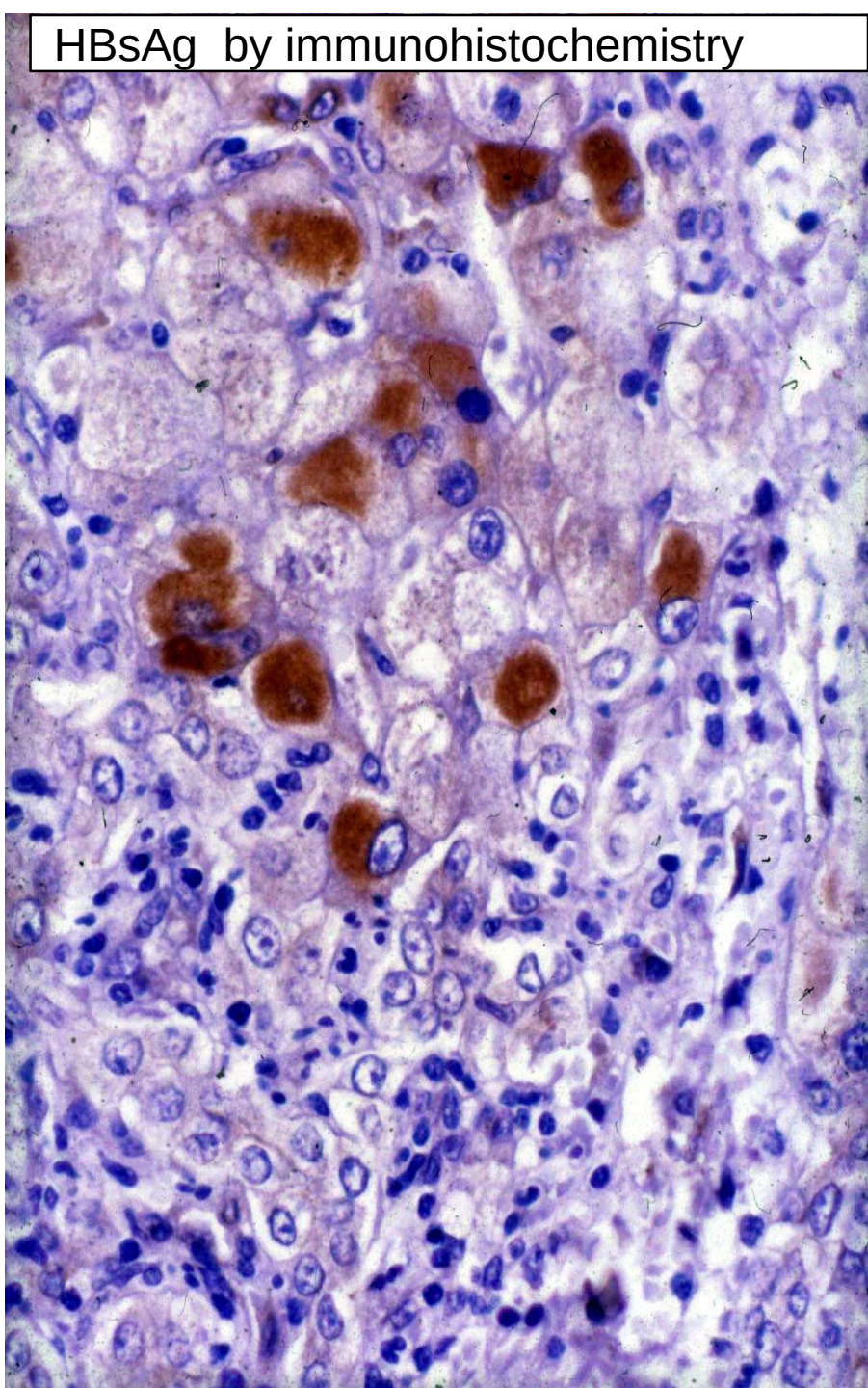
10%
Chronic inflammation

Cirrhosis

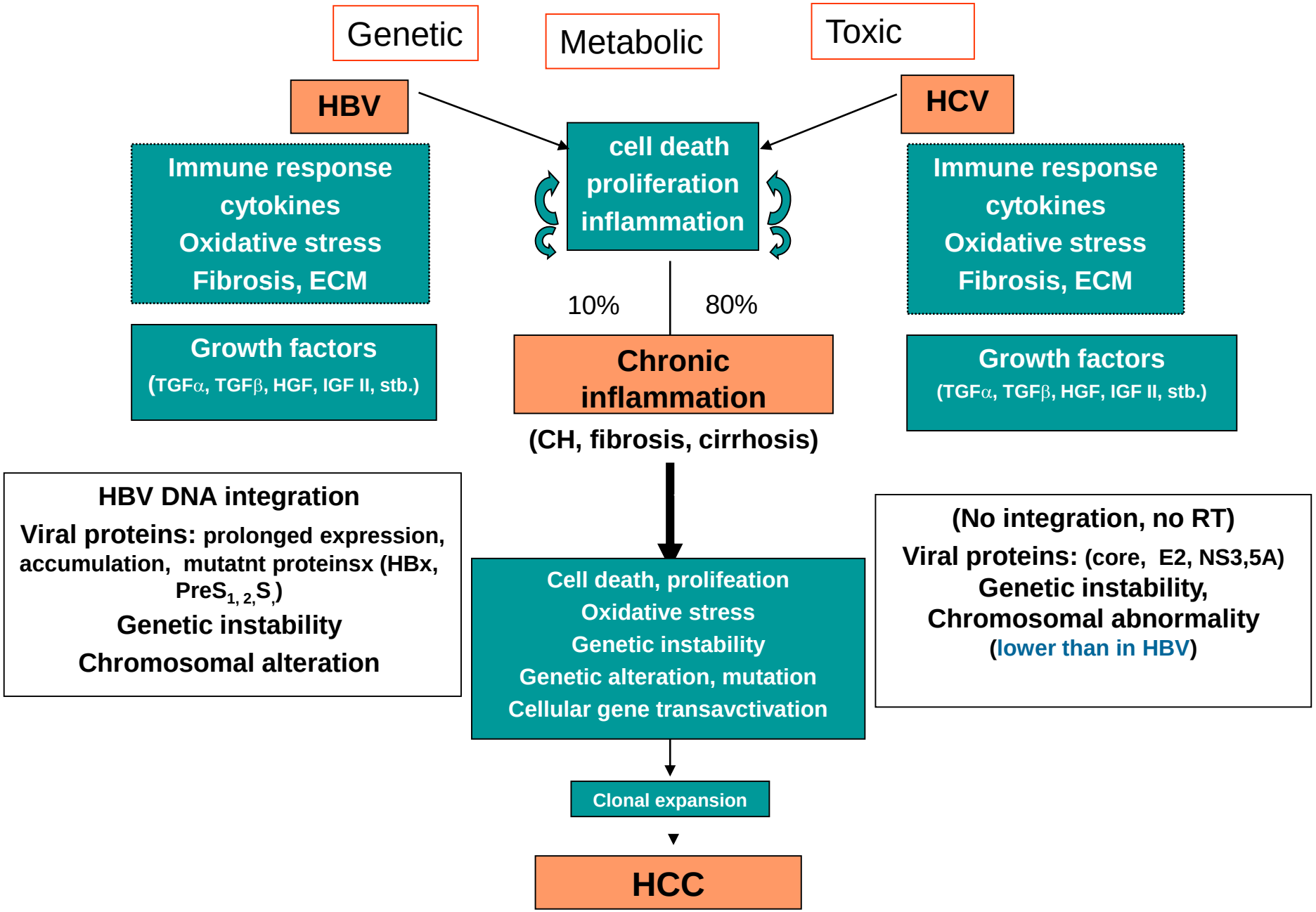
Liver cancer



Schaff et al. Antiviral Therapy 3:117-131 1996

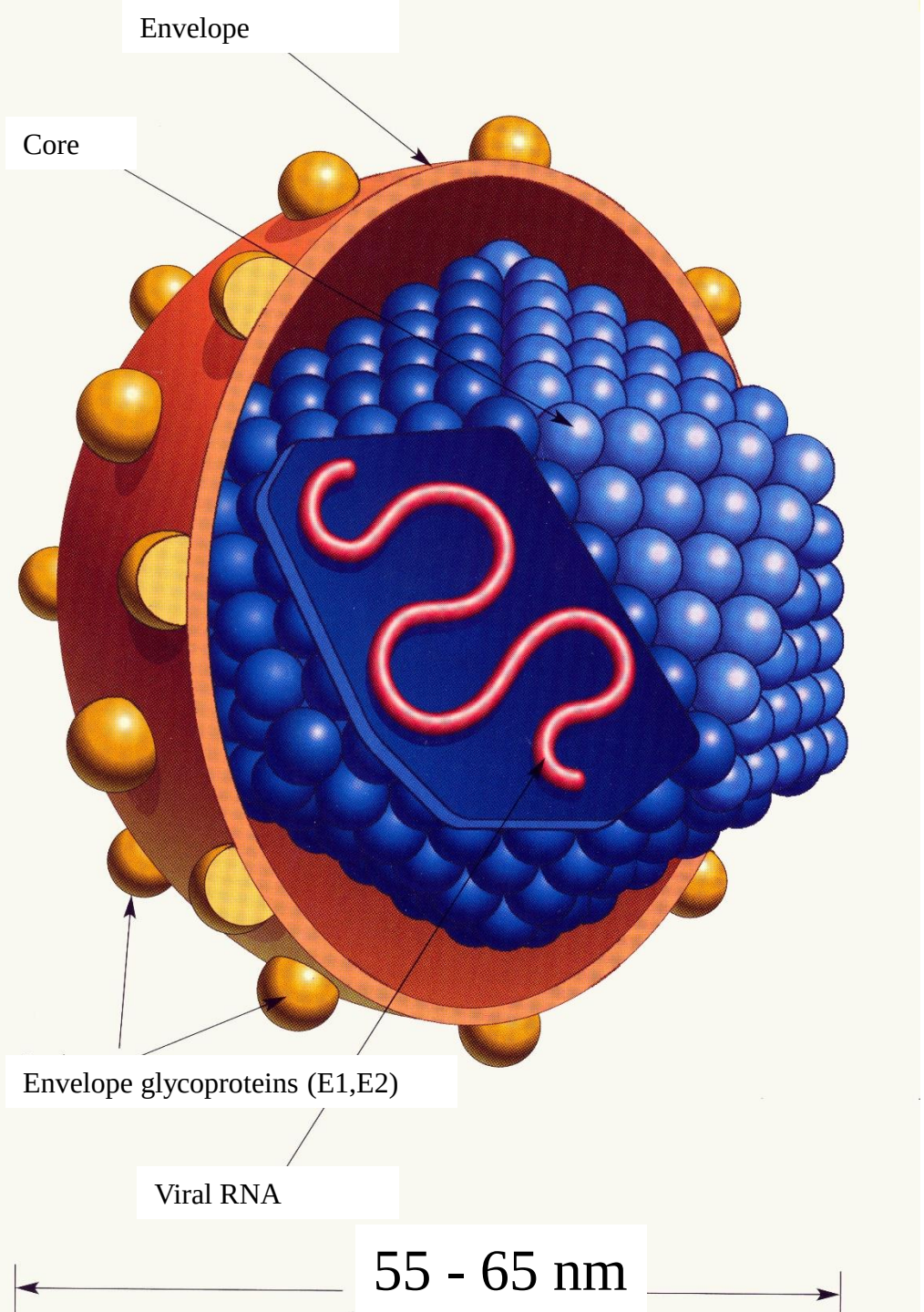


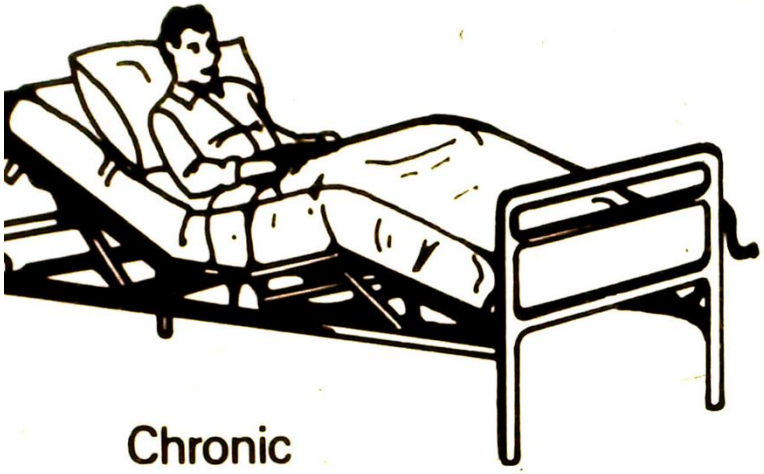
Pathomechanism of virus-induced HCC



HCV

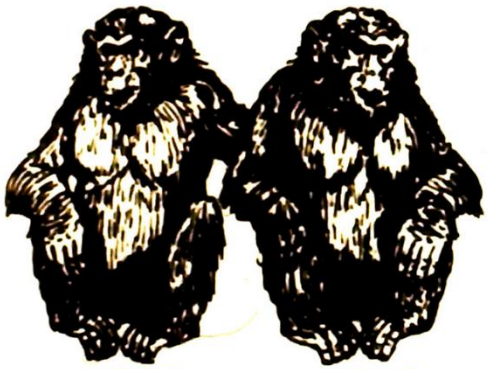
(**)


Envelope
Core
Envelope glycoproteins (E1,E2)
Viral RNA
55 - 65 nm



Chronic
Non-A, Non-B Hepatitis

Broken Pipette



#922 #930

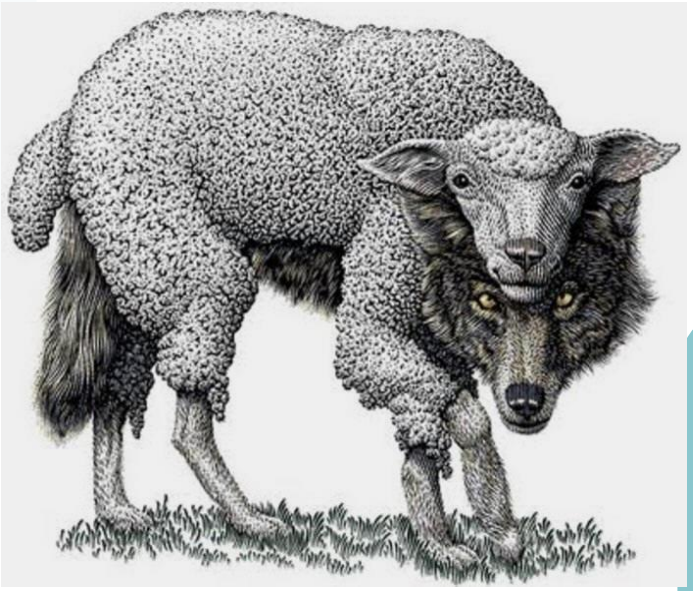
Non-A,Non-B hepatitis

Lancet 1978 8062: 463-466

HCV: „Bárányбőrbe bújt farkas”

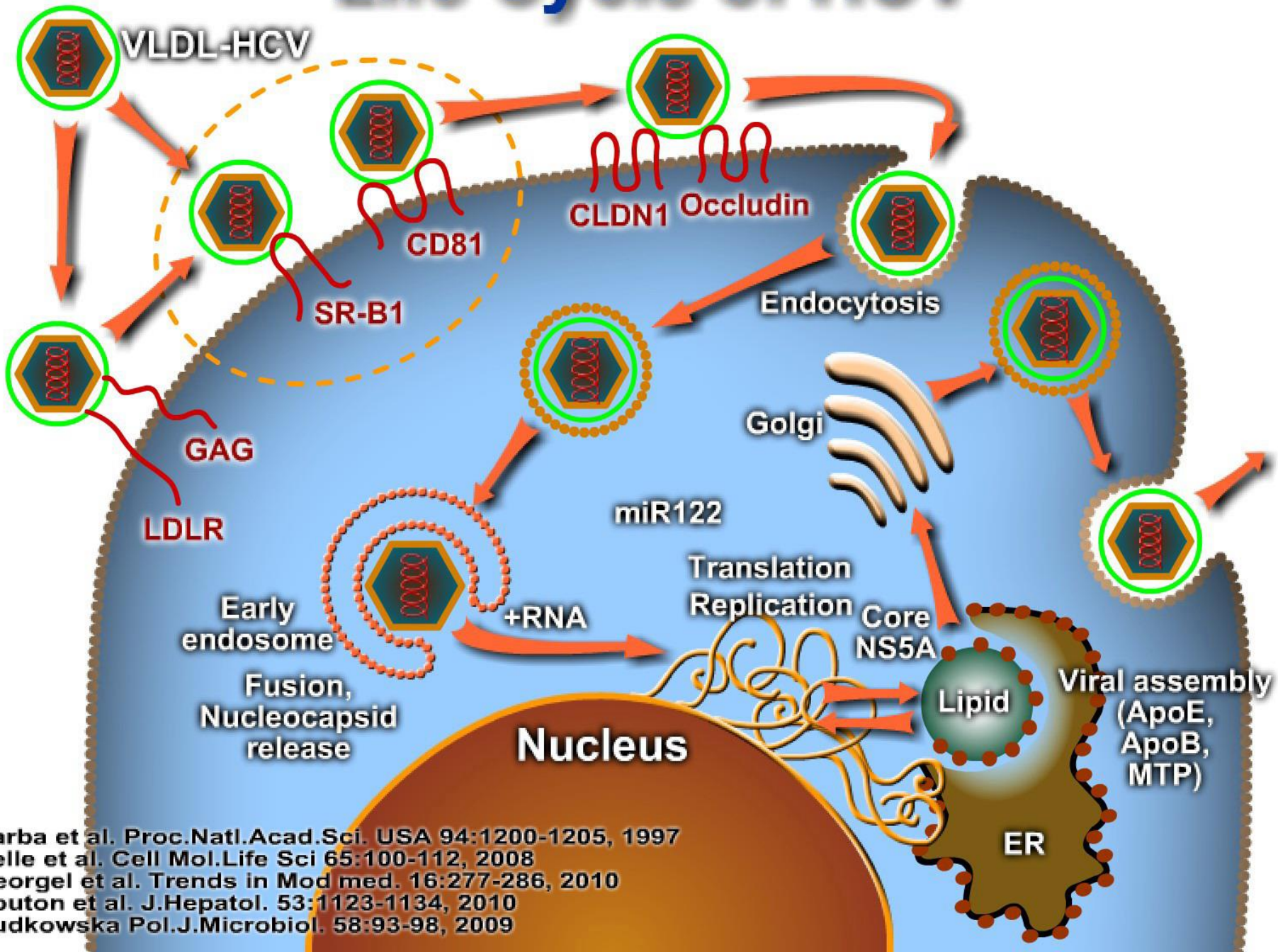
(„wolf in lamb’s clothing – wolf in sheep’s clothing”)

- Milder and more prolonged (15-30 yrs)
- Transmission: transfusion, sexual (rare), perinatal (rare)



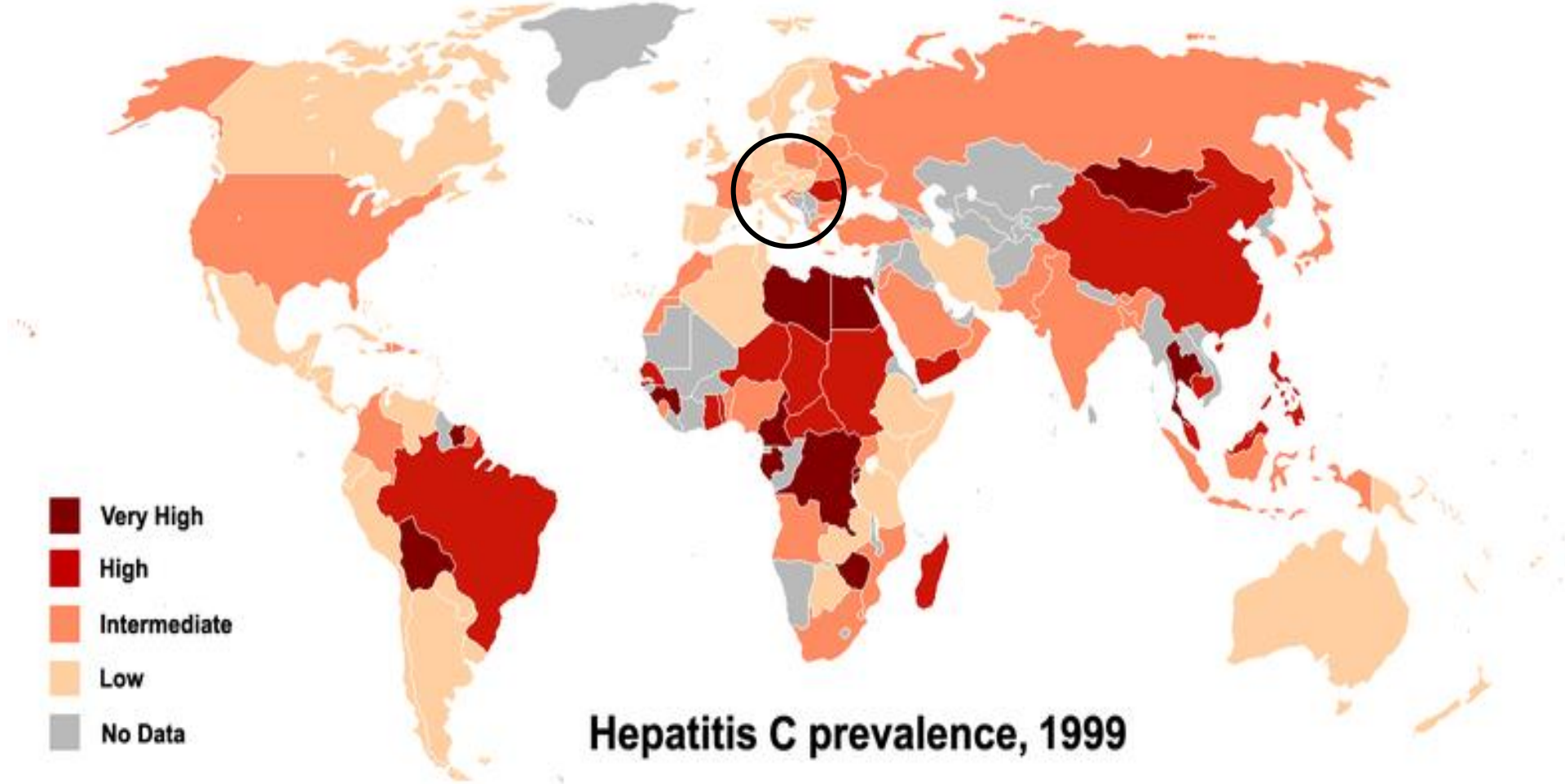
- 71 million infected
- **80%** progression into chronicity
- No vaccine

Life Cycle of HCV

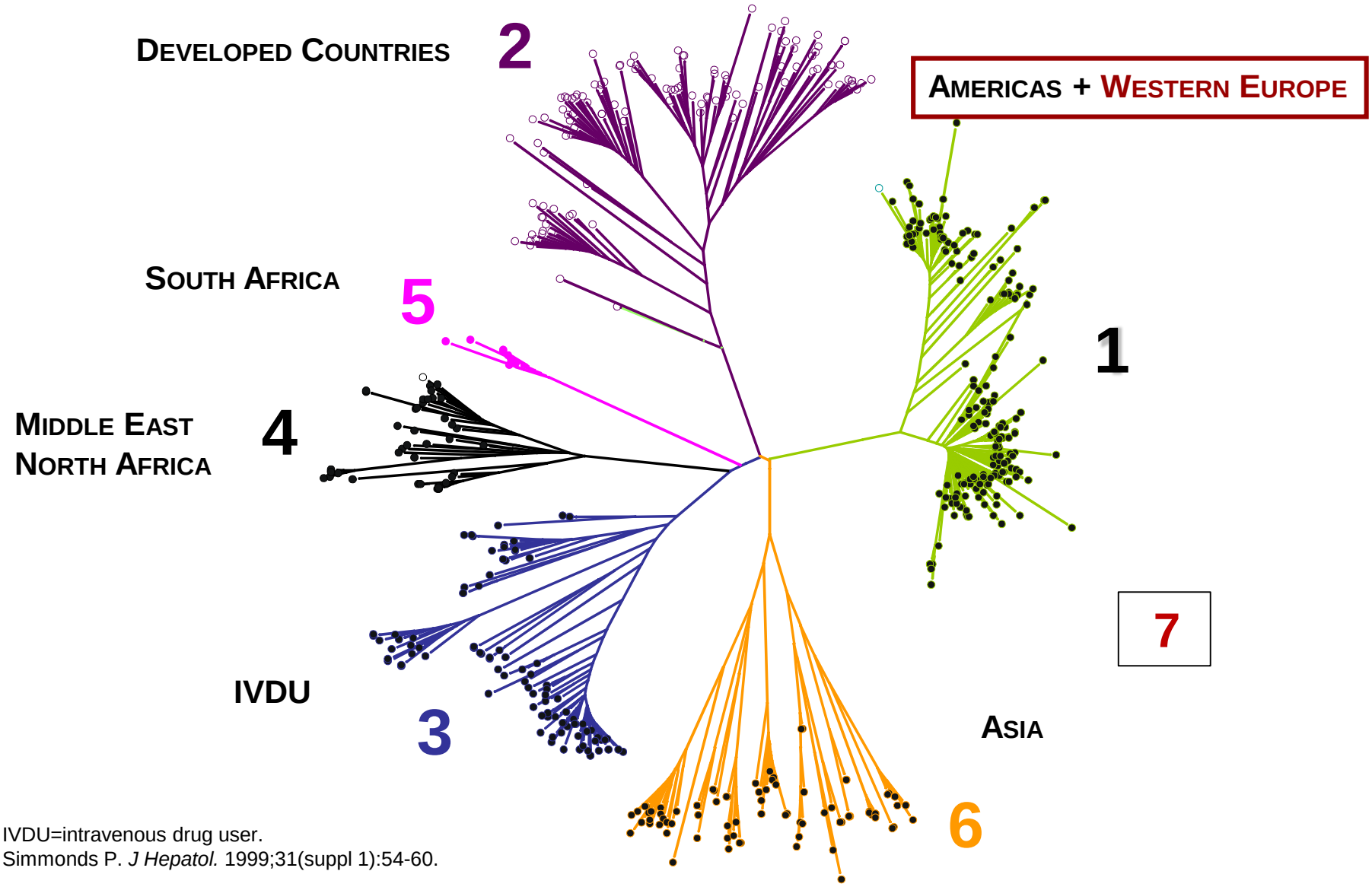


Barba et al. Proc.Natl.Acad.Sci. USA 94:1200-1205, 1997
Helle et al. Cell Mol.Life Sci 65:100-112, 2008
Georgel et al. Trends in Mod med. 16:277-286, 2010
Routon et al. J.Hepatol. 53:1123-1134, 2010
Budkowska Pol.J.Microbiol. 58:93-98, 2009

Worldwide Prevalence of HCV



HCV Genotypes

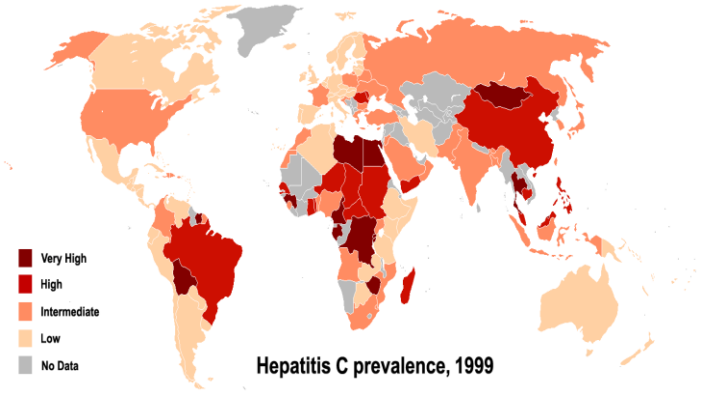


IVDU=intravenous drug user.

Simmonds P. *J Hepatol.* 1999;31(suppl 1):54-60.

Prevalence of HCV infection

Chronic infection: **71** million, **1%** of world population

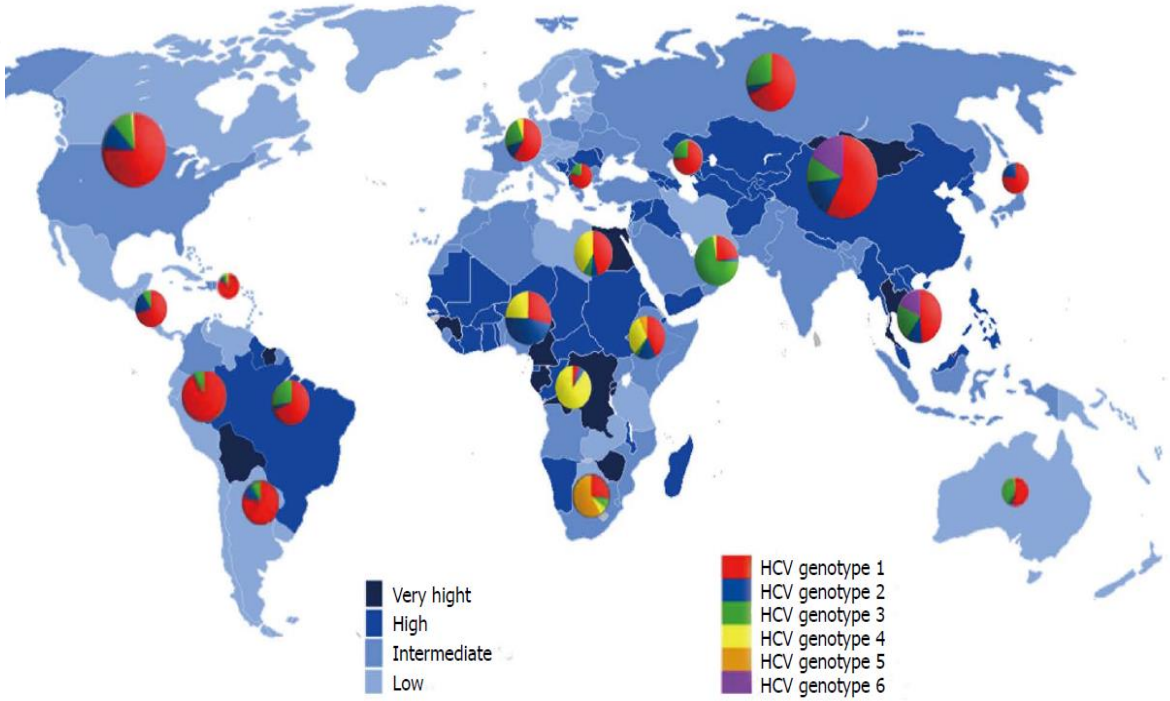


Highest: *Egyiptom (14,7%)*

East-Middle Europe

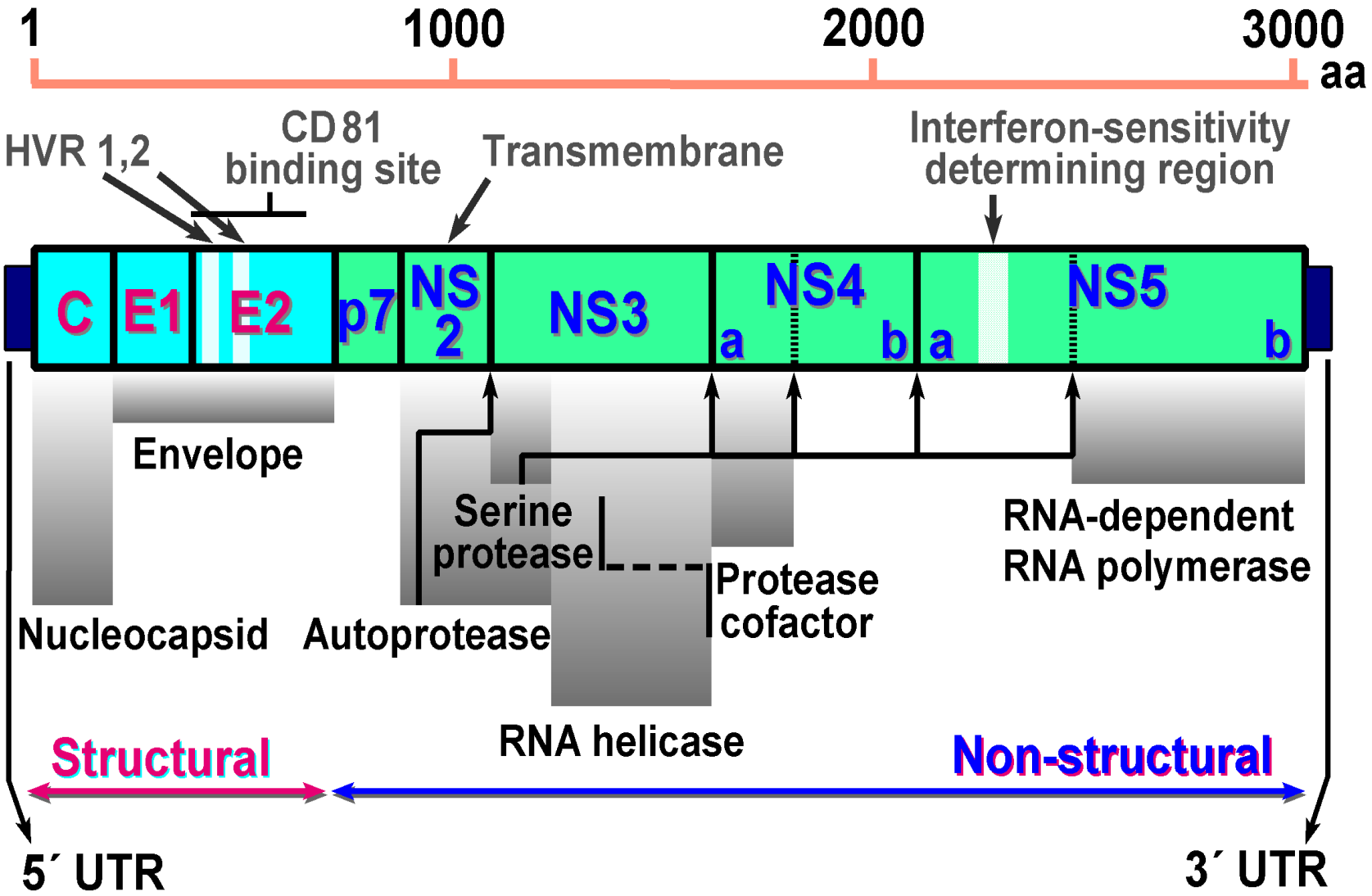
Low (0,2-1,0%): Cseh, Albania, Croitia, **Hungary**

- **Middle (1,4%):** Poland, Bulgaria
- **High (2,3-3,3%):** Ukraina, Romania

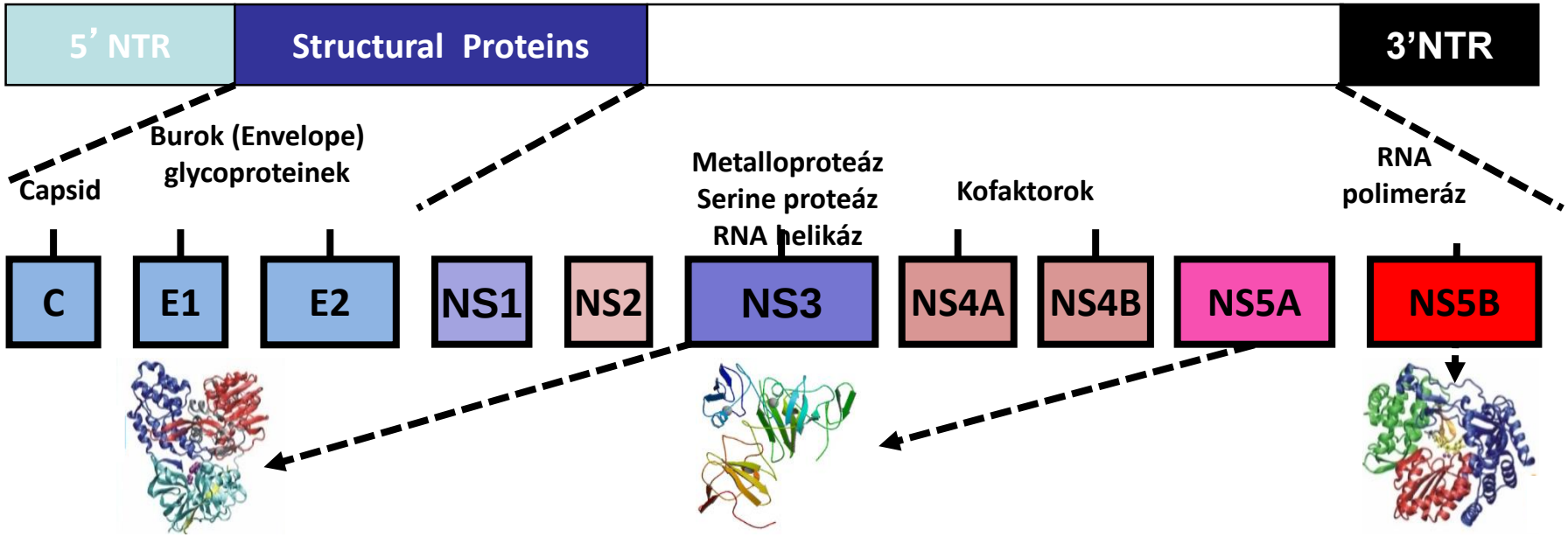


WHO Global Hepatitis Report 2017
Daw et al. WJV 5: 170-182, 2016

Structure of HCV genome

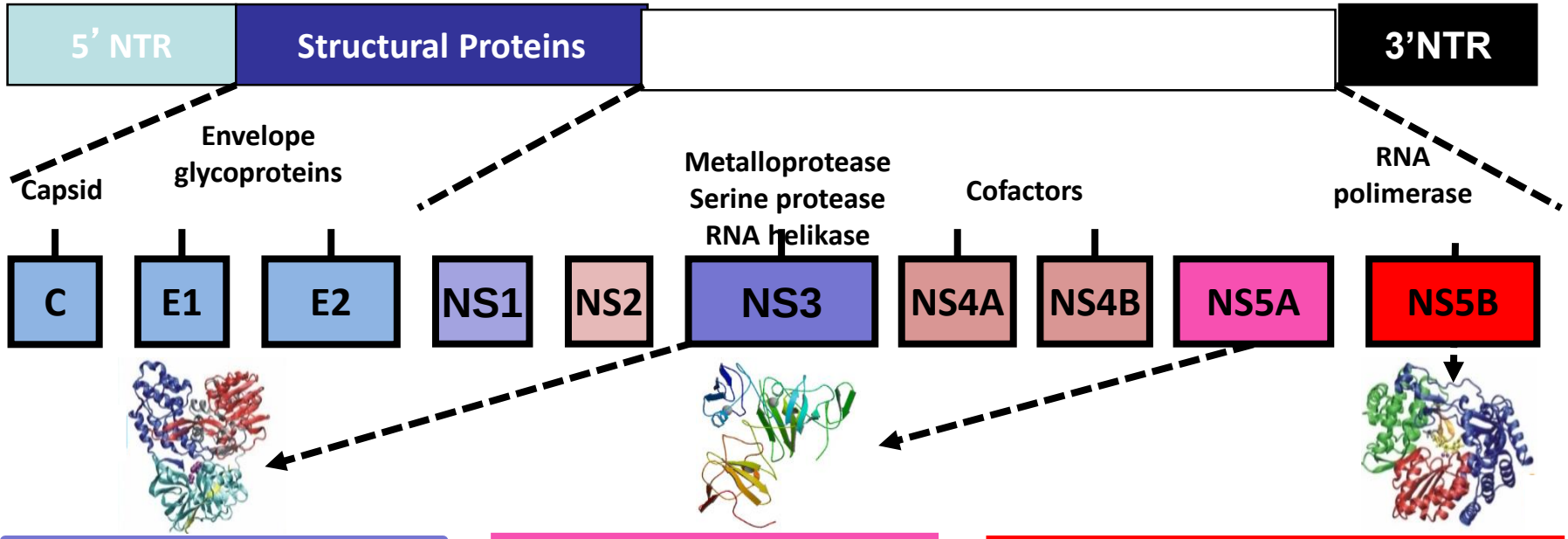


Direct-acting antivirals, DAAs



- Adapted from Schinazi R, *et al. Liver Int* 2014; **34** (Suppl 1):69–78.

Direct-acting antivirals, DAAs



Protease inhibitors

Paritaprevir (ABT-450)

Telaprevir

Boceprevir

Simeprevir

Faldaprevir

Asunaprevir

MK-5172

Sovaprevir

ACH-2684

NS5A inhibitors

Ombitasvir

ABT-267

Daclatasvir

Ledipasvir

GS-5816

ACH-3102

GSK-2336805

Samatasvir

MK-8742

Polimerase inhibitors

Nucs

Sofosbuvir

VX-135

IDX-20963

ACH-3422

GS-9669

TMC-647055

Non-Nucs

Dasabuvir

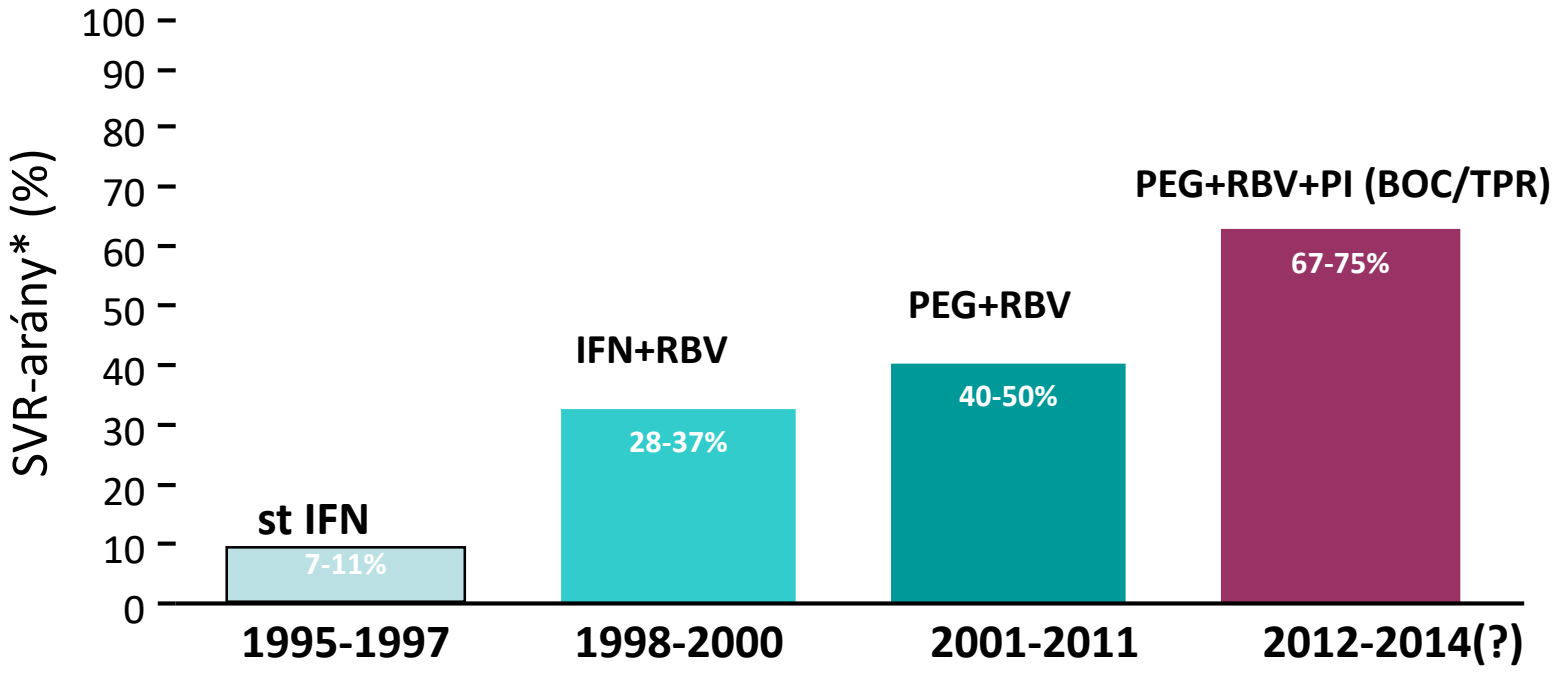
Deleobuvir

BMS-791325

PPI-383

- Adapted from Schinazi R, *et al. Liver Int* 2014; **34** (Suppl 1):69–78.

Steps in treatment of chronic hepatitis C
Naiv patients



*G1 HCV fertőzött, terápianaiv betegek

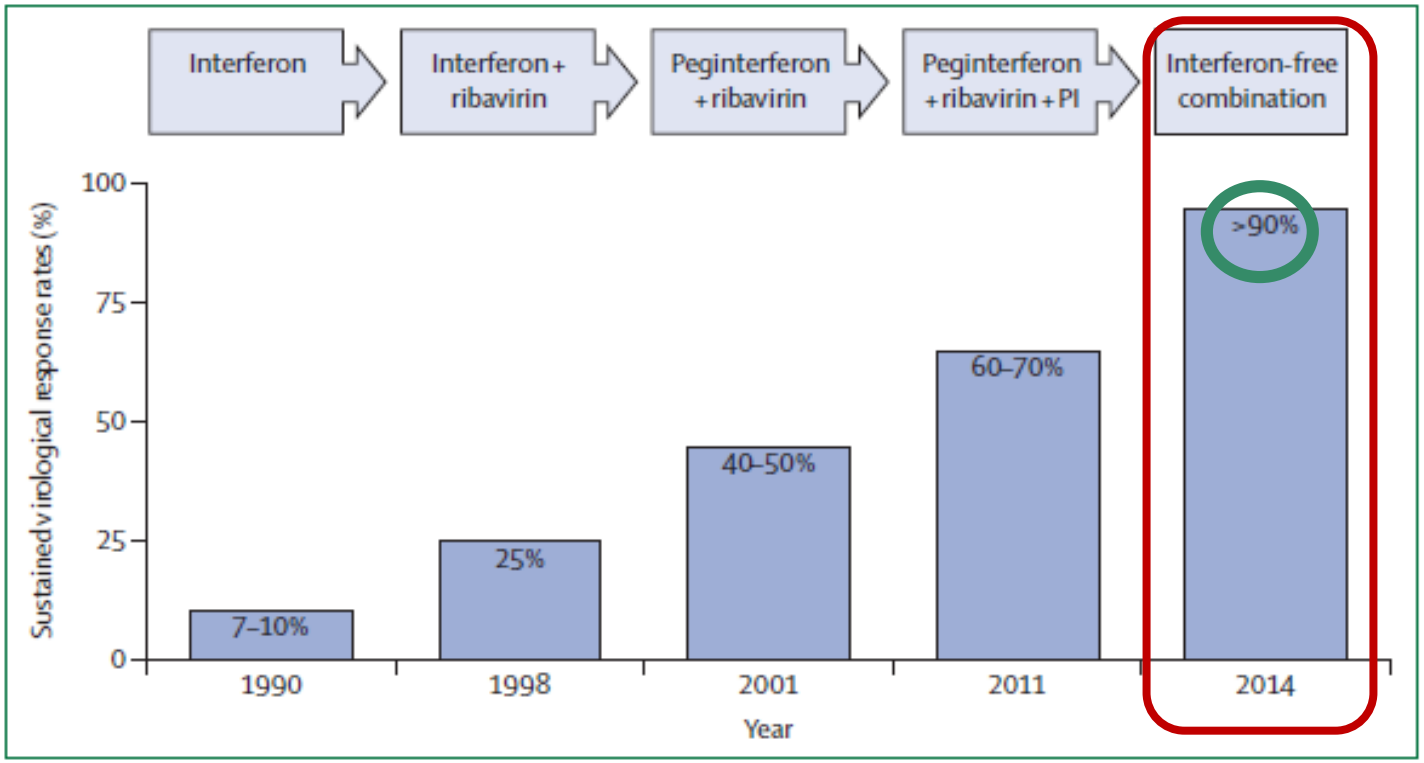
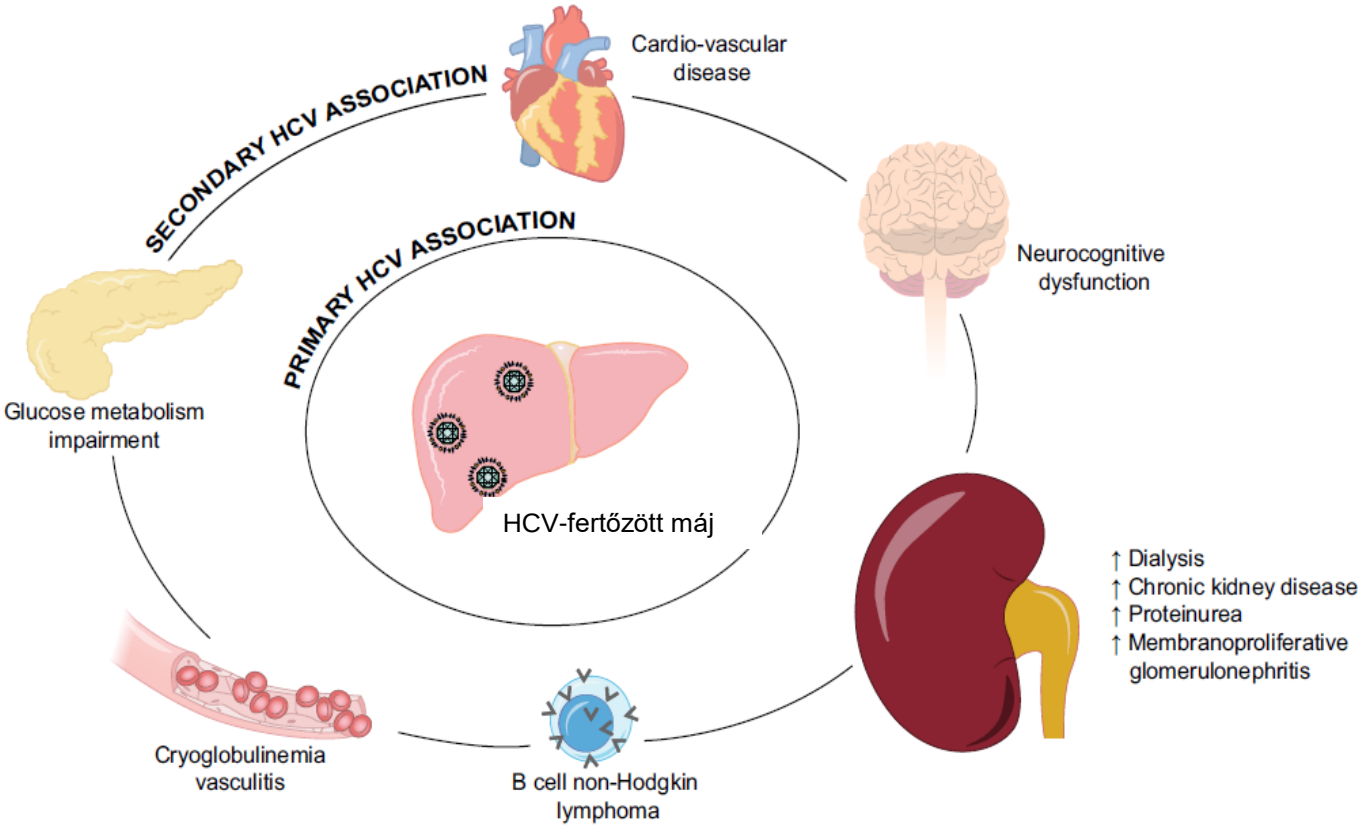


Figure 1: **Changes in standard of care for HCV, and improvements in numbers of sustained virological responses**
Data from references 9–12. PI=protease inhibitor.

Webster et al. Lancet Febr.14. 2015.

Manifestations of chronic HCV infection



Cacoub P, et al. J Hepatol, 2016

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

58:1045-46, 2013

Hepatology Snapshot

Hepatitis E virus infection: Multiple faces of an underestimated problem

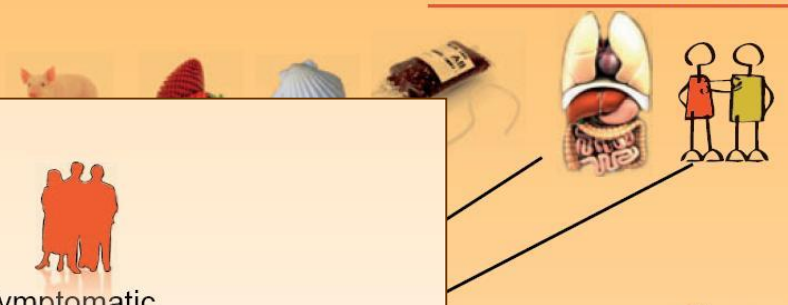
Sven Pischke, Heiner Wedemeyer*

Medizinische Hochschule Hannover, Hannover, Germany

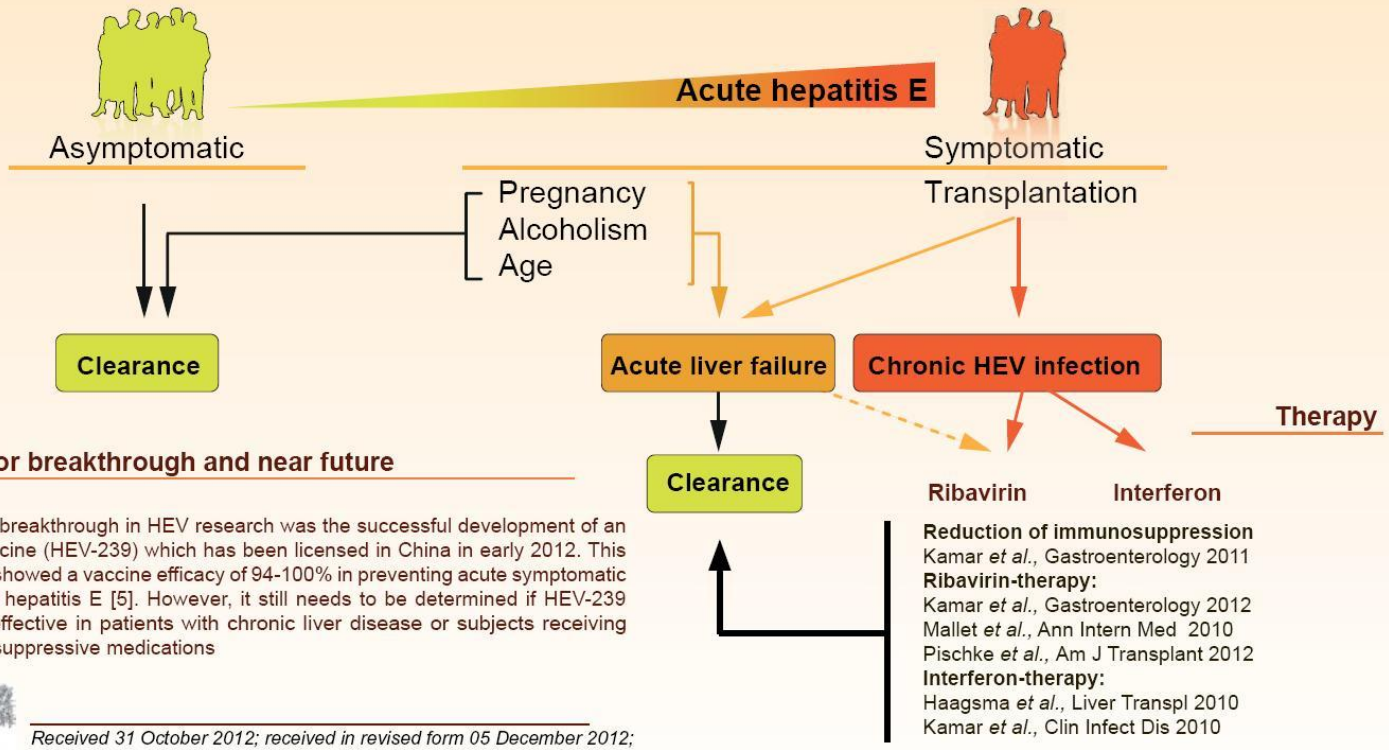
Key facts

- HEV is a relevant clinical problem also in industrialized countries
- In addition to waterborne infections, zoonotic and blood-borne

Transmission



Clinical course of exposed individuals



Diagnosis

is E is frequently diagnosed by the
n of HEV antibodies followed by testing for
NA in blood or stool. However, serological
show a wide variability in sensitivity and
ty and HEV antibodies may be negative
nocompromised individuals. Thus, direct
or HEV RNA is advised in all patients [1]

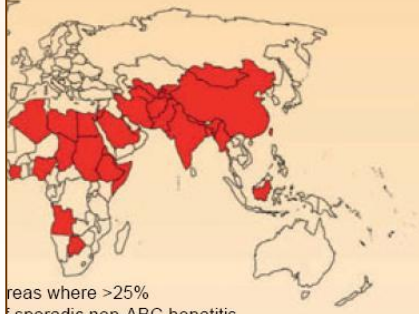
Therapy

Ribavirin **Interferon**

Reduction of immunosuppression
Kamar *et al.*, Gastroenterology 2011
Ribavirin-therapy:
Kamar *et al.*, Gastroenterology 2012
Mallet *et al.*, Ann Intern Med 2010
Pischke *et al.*, Am J Transplant 2012
Interferon-therapy:
Haagsma *et al.*, Liver Transpl 2010
Kamar *et al.*, Clin Infect Dis 2010

Major breakthrough and near future

A major breakthrough in HEV research was the successful development of an HEV vaccine (HEV-239) which has been licensed in China in early 2012. This vaccine showed a vaccine efficacy of 94-100% in preventing acute symptomatic cases of hepatitis E [5]. However, it still needs to be determined if HEV-239 is also effective in patients with chronic liver disease or subjects receiving immunosuppressive medications



reas where >25%
sporadic non-ABC hepatitis
due to hepatitis E virus

Received 31 October 2012; received in revised form 05 December 2012; accepted 10 December 2012

Epidemiology

- 1st cause of acute hepatitis
  - Worldwide, in Europe, in France

    - 20 million cases /year
    - 70 000 death/year
    - > 3 million symptomatic patients

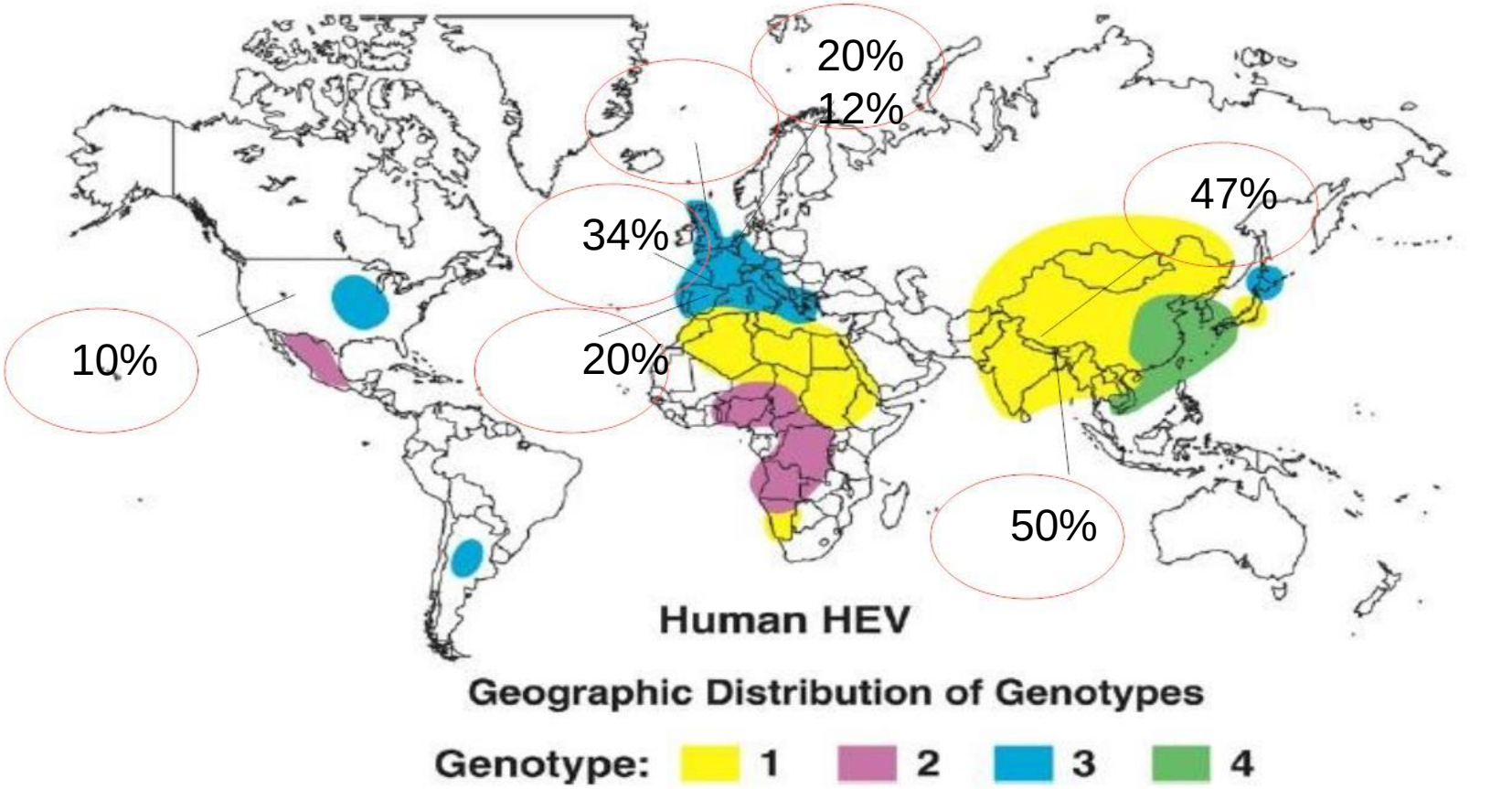
- In Europe: 2 million cases/year

WHO. Viral hepatitis. Jul, 2015
EASL guidelines 2018 vol 68 1256

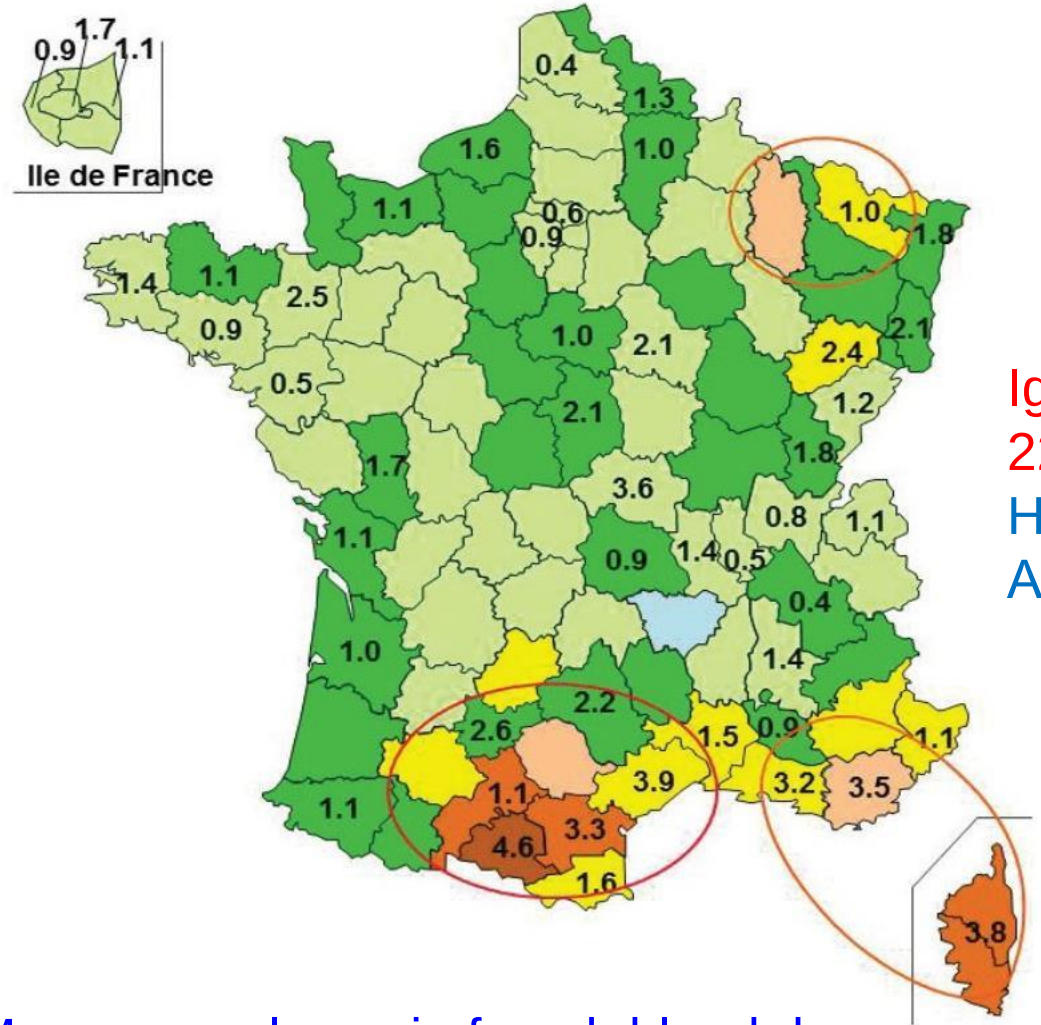
Comparison of HEV genotypes

Characteristcs	HEV 1 and 2	HEV3 and 4
Source of infecton	Obligate human pathogen	Zoonotic Blood supply
Route of □ifecton	Faecal-oral via infected water	Consumpton of infeted pork Blood supply
Outbreaki	Yes	No
Clinical attack rate	1:5	< 1:10
Demographics	Mainly affecting young adults	Mainly affecting older men Male:female rato 3:1
Chronic □nfecton	No	Yei in □imunosuppressed □individualis
Occurrence of second HEV infecton	Yes	Yes
Neurological sequelae	Yes	Yes

Seroprevalence



Stramer SLTransfusion 2015
Izopet J Clin V□rol 2015
Dalton H Curr Infec□Dis Rep 2014



IgG seroprevalence :
22,4 % in France
Haute Loire : 8%
Ariège : 86,4%

IgG and IgM seroprevalence in french blood donor

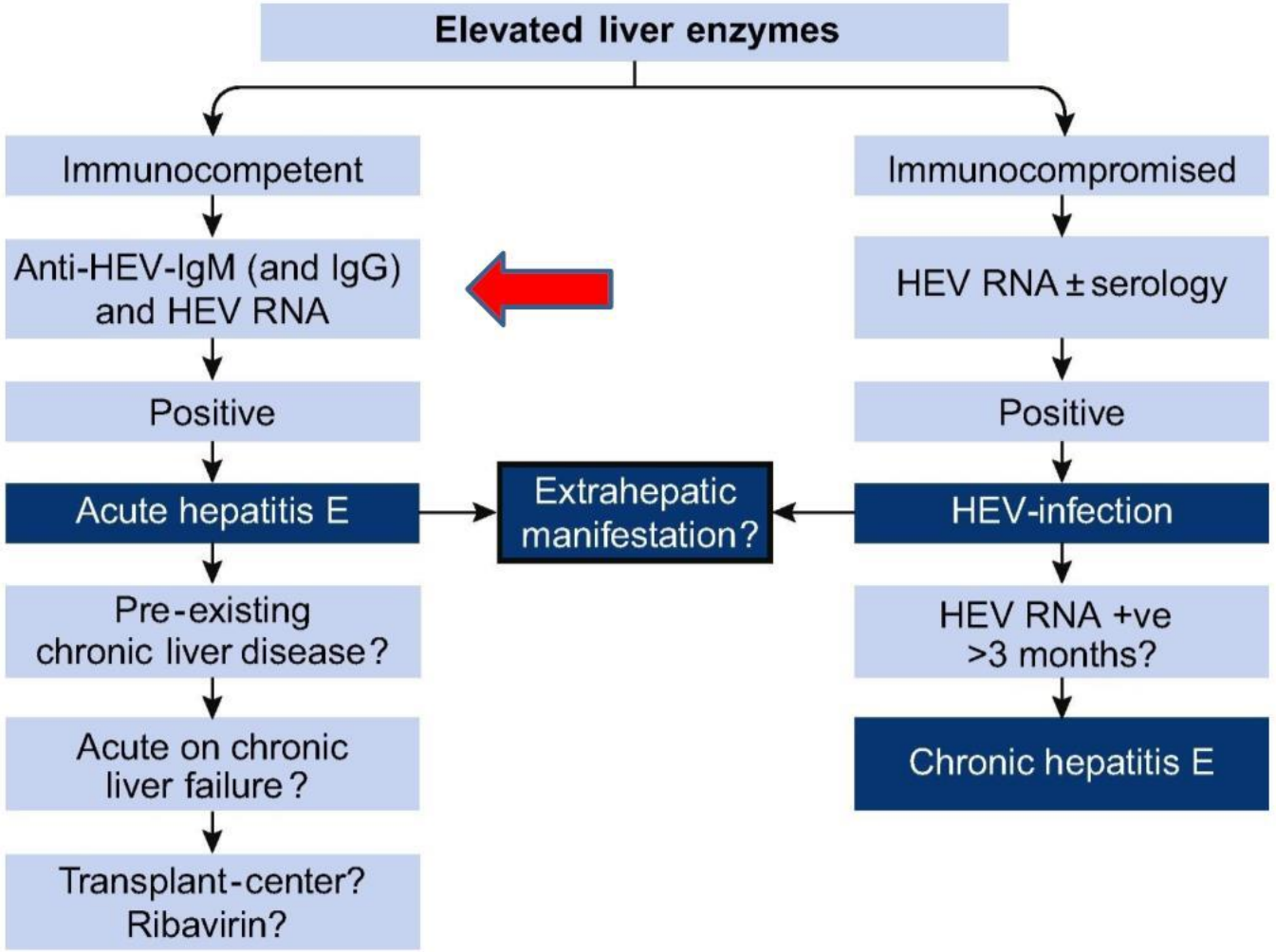
Blue < 10%, green 20-30%, yellow 30-40%, pink 40-50 %
Orange 60-70%, brown > 70%

Maniuy e al Hepatology 2016

When should you look for HEV?
EASL guidelines

- Acute viral hepatitis (A1)
- Suspected drug induced liver injury (A1)
- Decompensated cirrhosis
  - Guillain-Barré syndrome, neuralgic amyotrophy(B1),
  - Acute neurological symptoms associated with elevated transaminases
- Elevated transaminases following transfusion (A1)

HEV diagnosis
EASL guidelines



EASL | JOURNAL OF HEPATOLOGY

Conclusion 1

- HEV infection diagnosis is rising
- Subtype 3 may be more severe
- Neurological symptoms are frequent (Neuralgic amyotrophy +++)

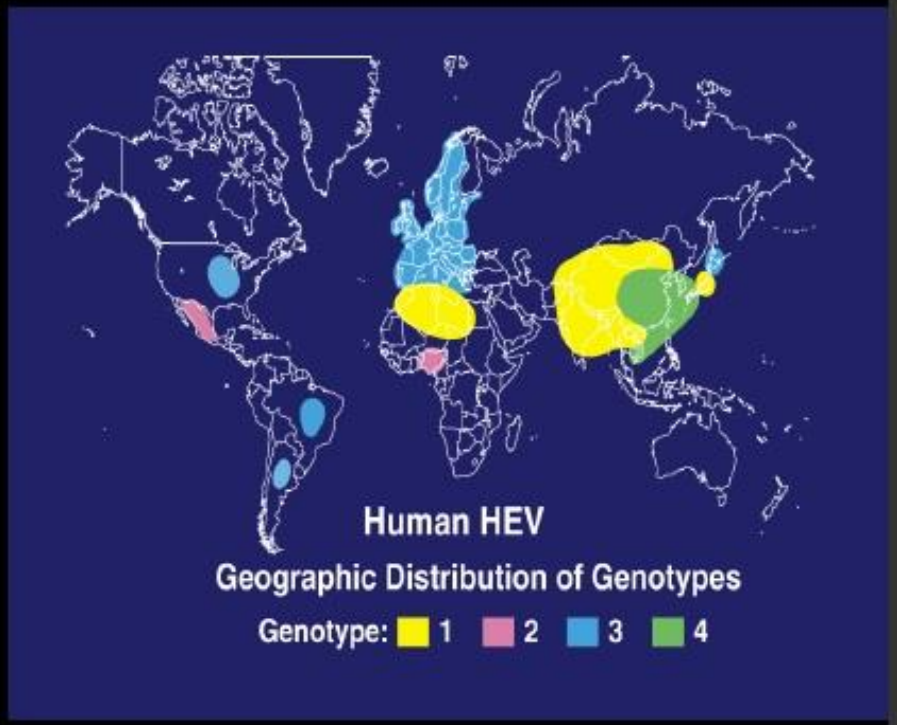
HEV: HISTORY

H.Alter HepDart, Dec.2013.

- 1978: Water-borne epidemic in Kashmir caused 20,000 icteric cases; 700 FH; 600 deaths; not HAV
- 1980: Epidemic hepatitis among Russian soldiers in Afghanistan; not HAV related
- 1983: Russian volunteer swallows fecal extract from 9 acute cases in the Afghan epidemic and recovers 27-30nm VLP from his acute phase stool (Balayan)
- CDC recovers identical VLP from macaques inoculated with acute phase stool; serial passage
- 1990: Bile from cyno macaques used in differential

HEV: VIROLOGY

- Small (32-34nm,7.2 kb), non-enveloped , positive-sense single-strand RNA virus in hepeviridae family (Hepevirus)
- 4genotypes; 1 serotype
- Gt 1,2: human virus endemic and epidemic predominantly in southeast Asia
- Gt 3,4: primarily swine viruses that infect humans (zoonosis)
- Gt3 main HEV infection in developed world; Gt4 in China



H.Alter HepDart, Dec. 2013

Genotype 1 vs 3 Hepatitis E

Feature	Genotype 1 (Epidemic)	Genotype 3 (Endemic)
Sex (M:F)	1:1	3:1
Age	20-45 yrs	40-80 yrs
2nd Spread	Uncommon	Not known
Source	Water	Food
Agent	Human	Swine
Seasonality	Yes	Usually not
Fatality rate	Pregnancy	Elderly
Extrahepatic	Yes (Pancreas)	Yes (CNS)
Chronicity	No	Yes, immune deficient

H.Alter HepDart Dec.2013

PARADIGM SHIFT IN PERCEPTION OF HEV INFECTION

Previously considered to be an acute, self-limited infection occurring only in endemic regions, it has now been shown to:

- Occur in surprisingly high frequency in non-endemic countries
- Evolve to chronic infection in immunosuppressed patients, particularly solid organ recipients
- Evolve to cirrhosis in those chronically infected
- Results in an asymptomatic chronic carrier state among immunosuppressed; undetermined if there is a carrier state in healthy, potential donors

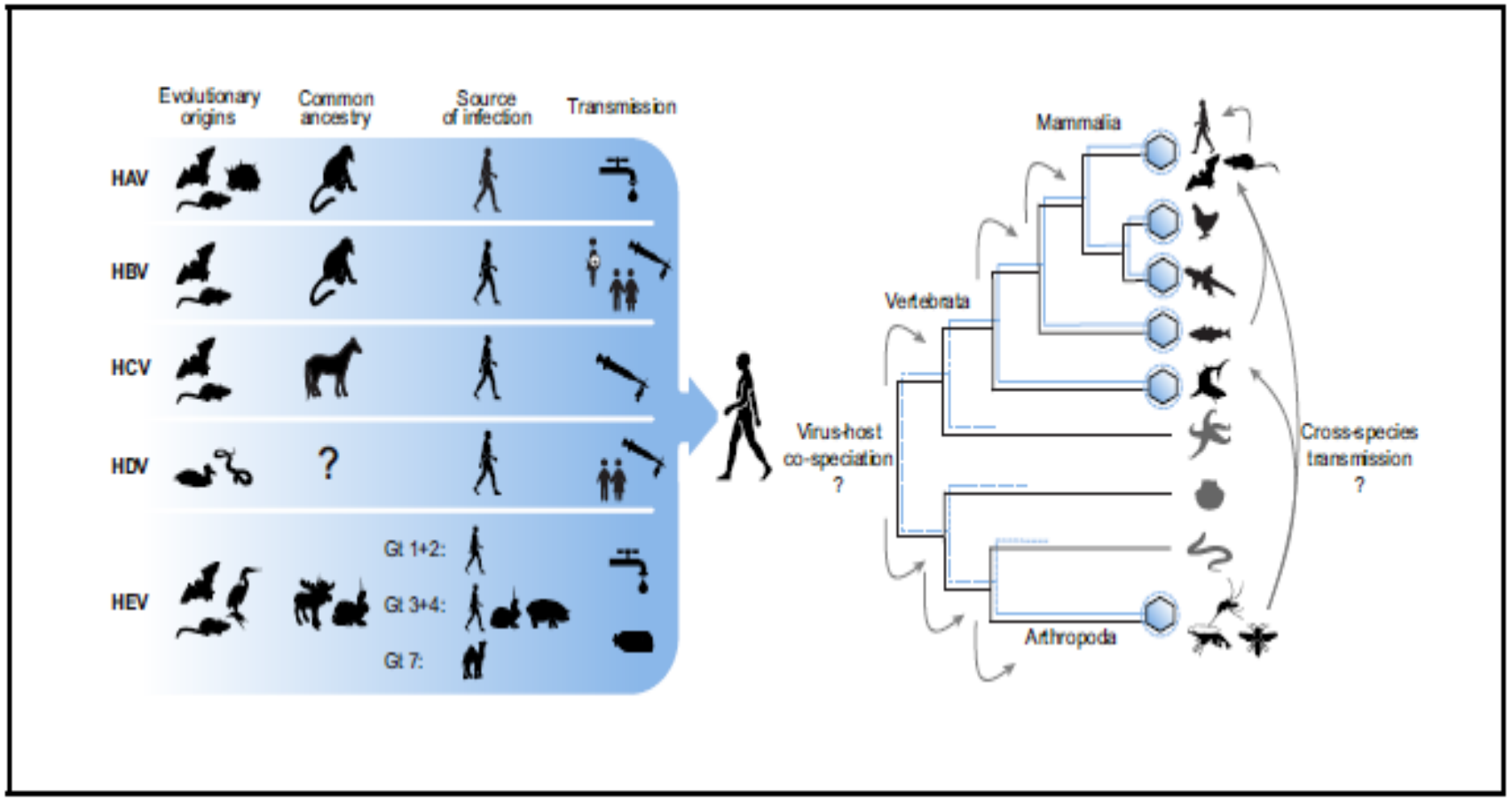
H.Alter HepDart, Dec. 2013

How Might Non-Swine Handlers be Exposed to Contaminated Pork?
[Caution: This slide is not for the queasy]

- Gastro Elitism Movement: wild boar pappardelli, pigs feet Milanese
- Figatelli (raw pork sausage) : favorite in Southern France
- Liver slime from pig poop is pooled and used to irrigate soil and plants

  (don't forget to eat your veggies)
- Dunkin Donuts sells pork donuts in China
- Scrapple made from pig heads and liver
- 11% of raw pig liver in US markets tested HEV RNA+
- USDA: cook pork meat to 145F; organs to 160F

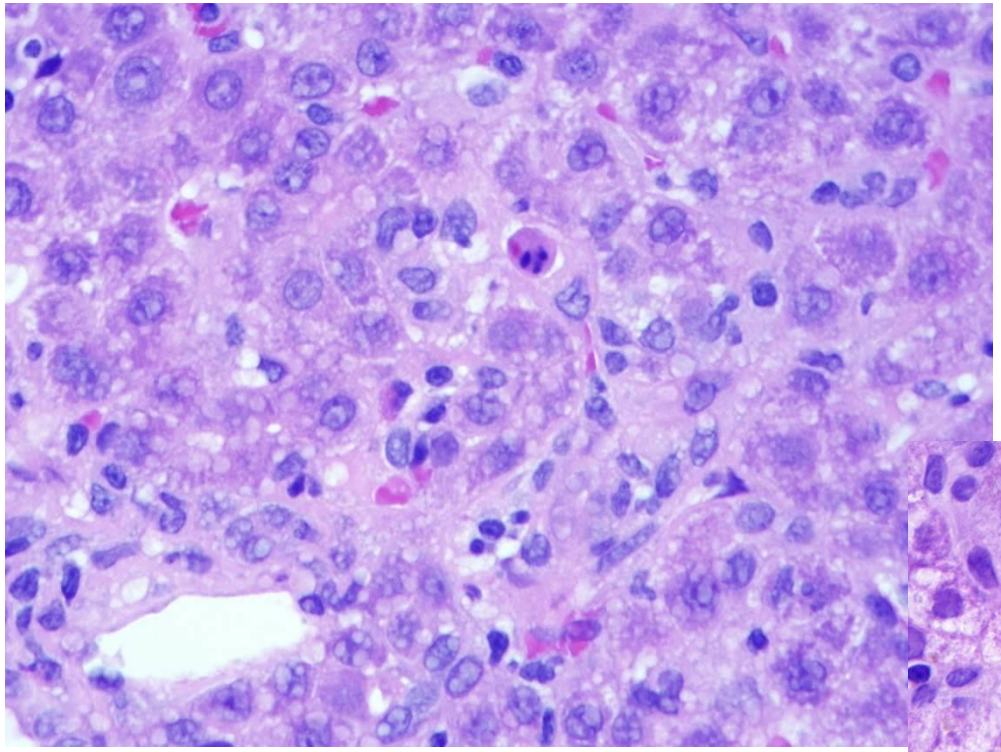
H.Alter HepDart, Dec. 2013

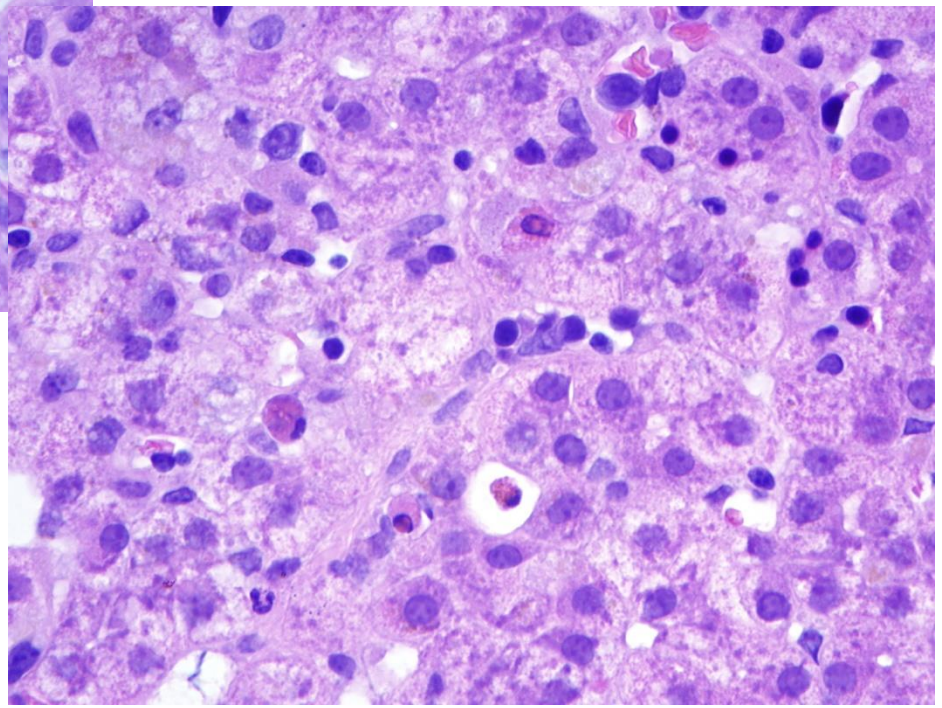

Evolutionary origins
Common ancestry
Source of infection
Transmission
HAV
HBV
HCV
HDV
HEV
?
Gt 1+2:
Gt 3+4:
Gt 7:
Mammalia
Vertebrata
Virus-host co-speciation ?
Cross-species transmission ?
Arthropoda

Pathology of acute viral hepatitis

- Gross: enlarged, reddish, or shrinken (extensive necrosis)
- Histology:
  - Lobular, portal and periportal inflammation
  - Inflammatory cells: dominating lymphos (but plasma cells, PMNs, eos in lower number)
  - Necrosis, apoptosis (Councilmann-like bodies)
  - Cholestasis (not always)
  - PAS+ Mas

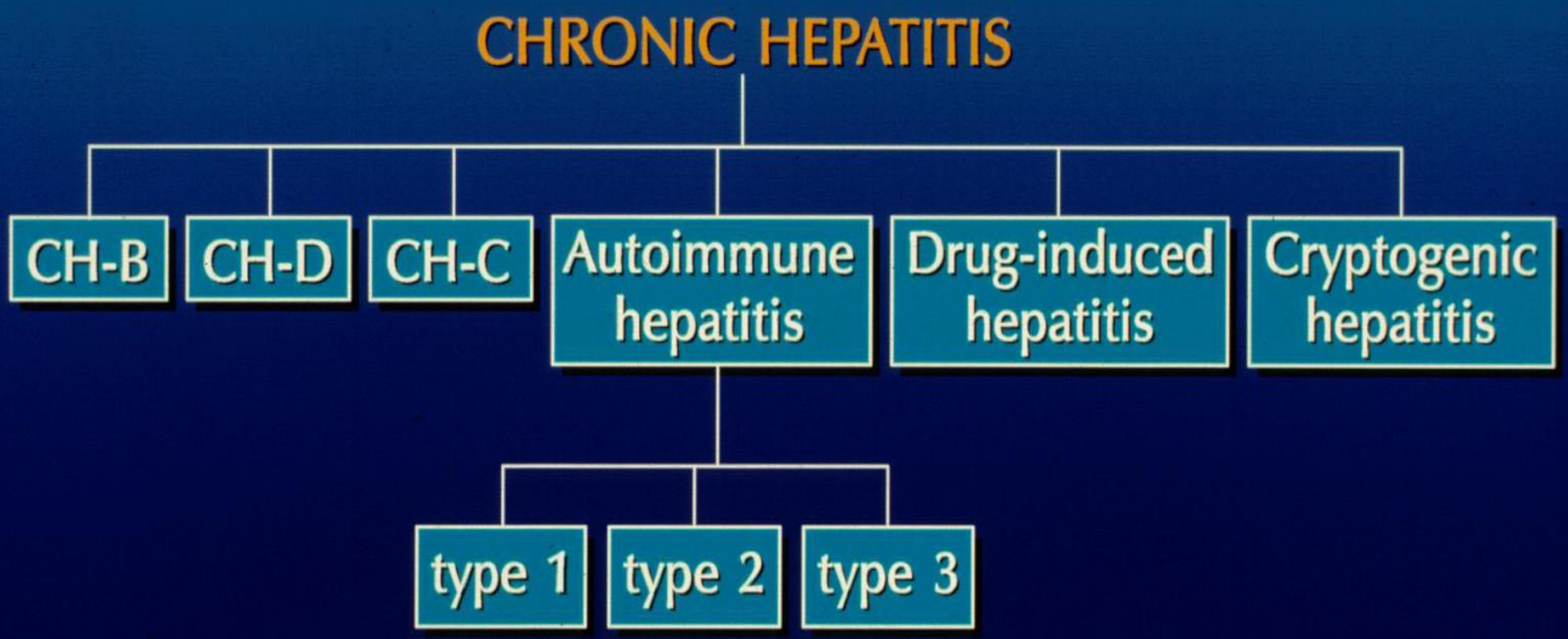
Akut vírushepatitis





Classification of Chronic Hepatitis

(XX)

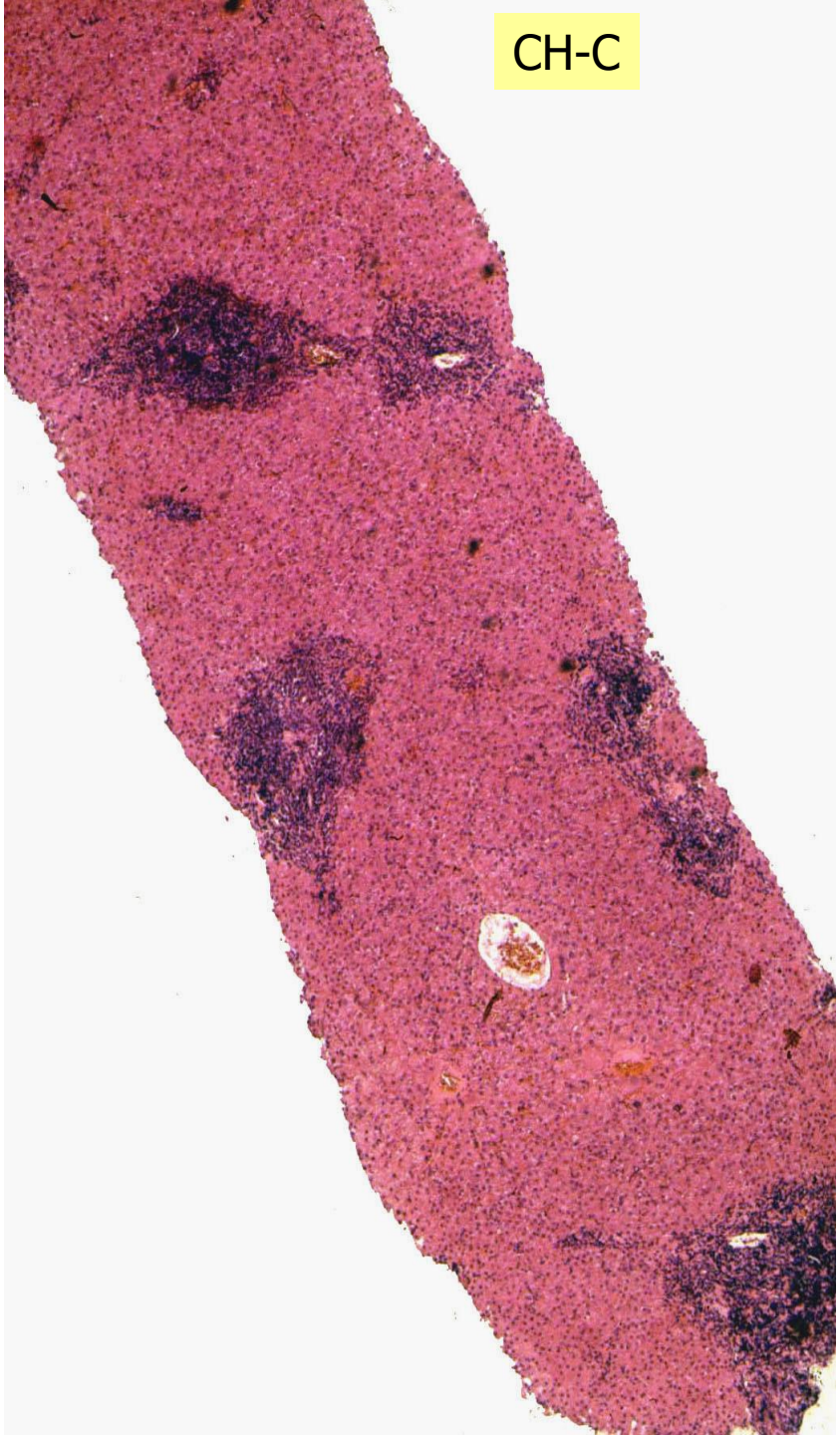


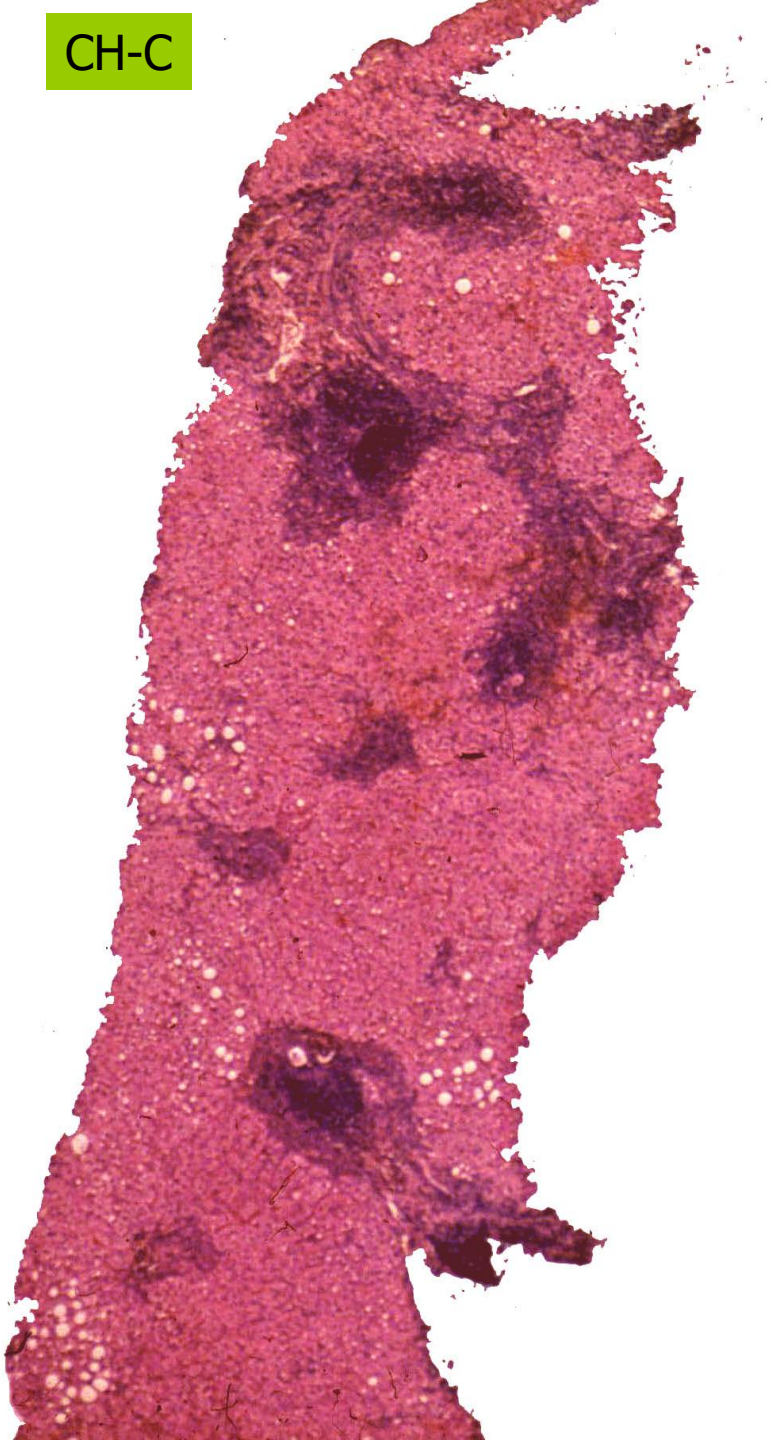
(Desmet et al., Hepatology 19:1513, 1994)

CDC

General pathomorphology of CH (**)

- necrosis / apoptosis
- inflammation
- fibrosis
- specific features
- Grading, staging


CH-C

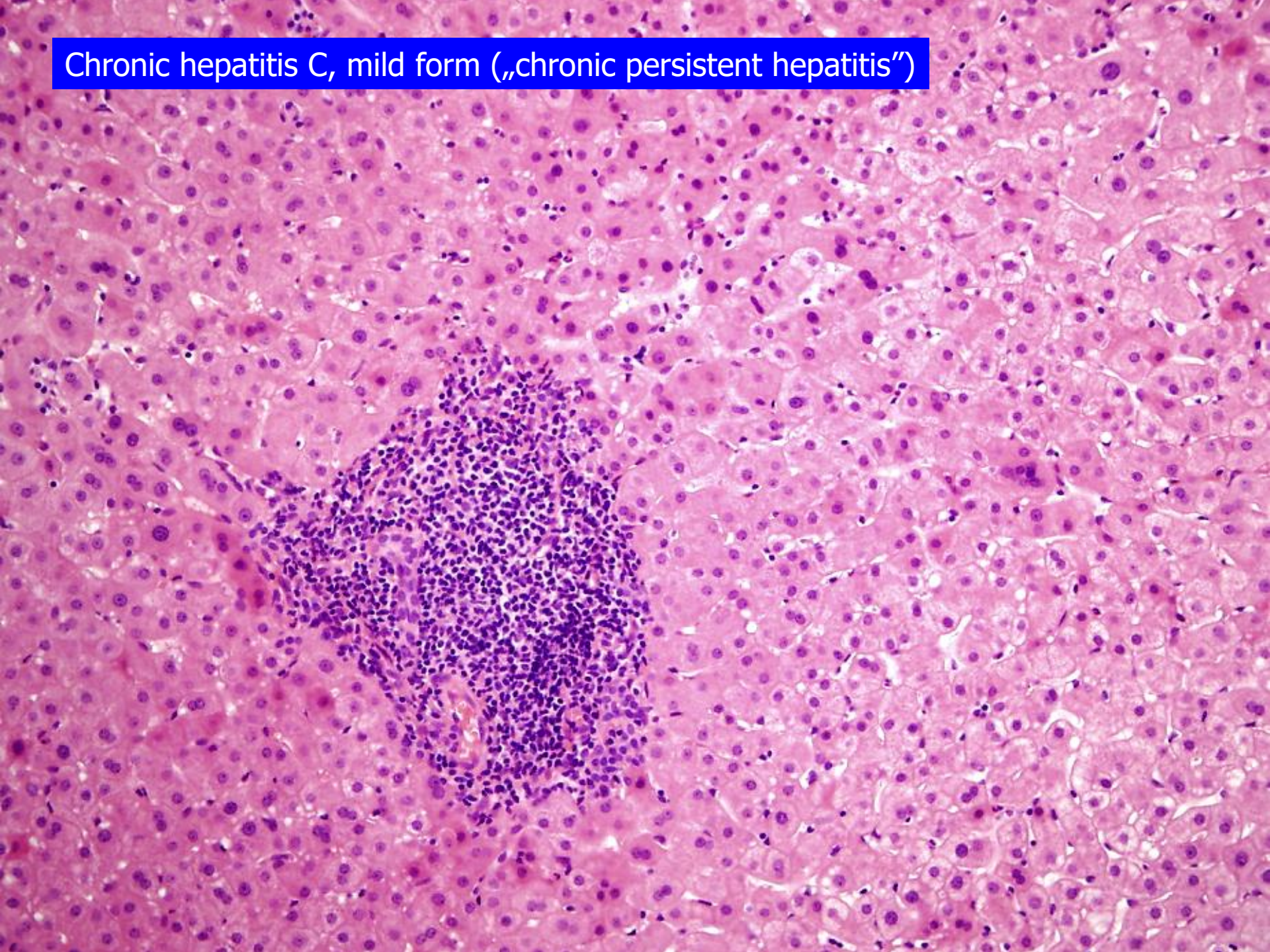

CH-C

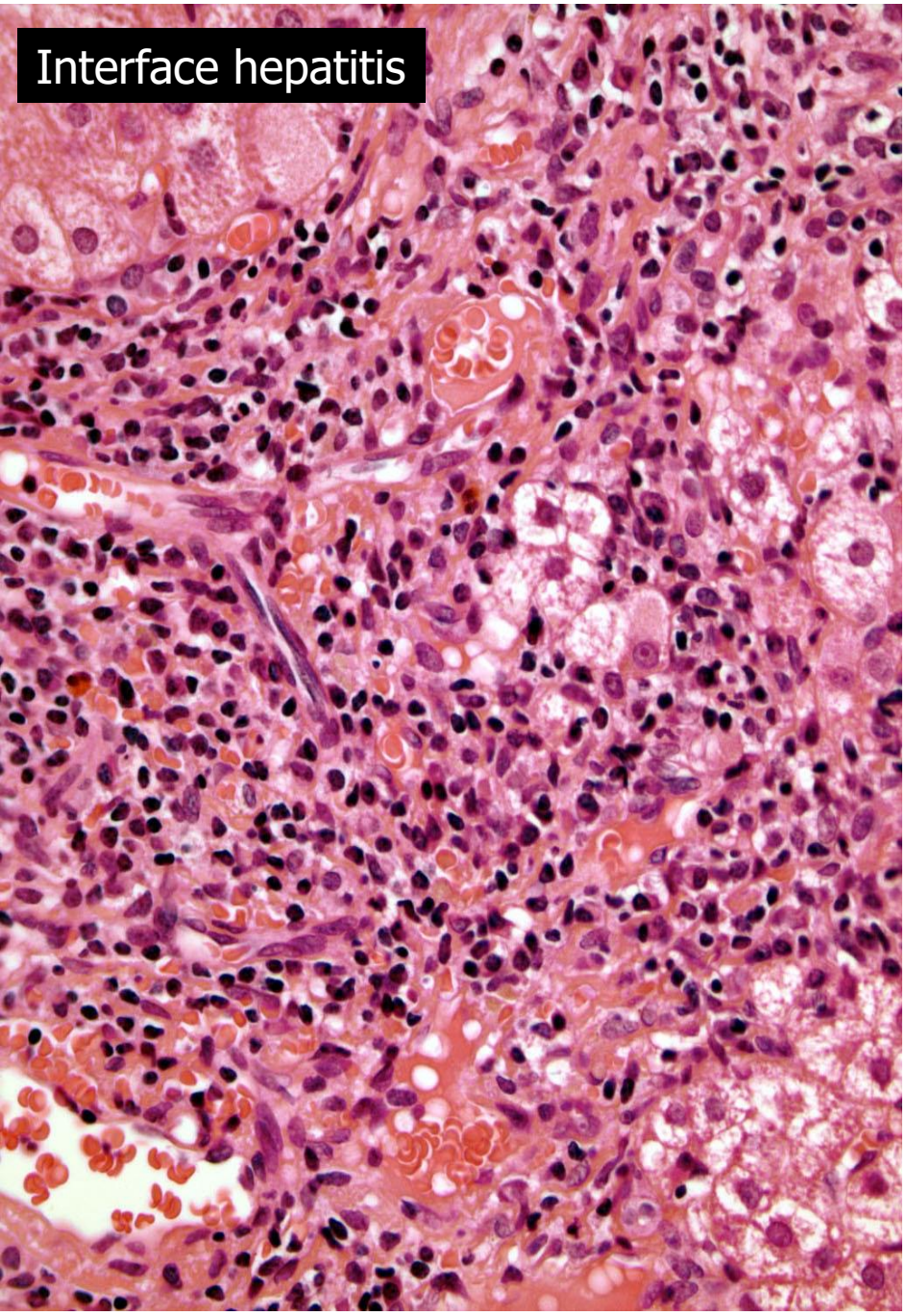
Histology Activity Index (HAI)

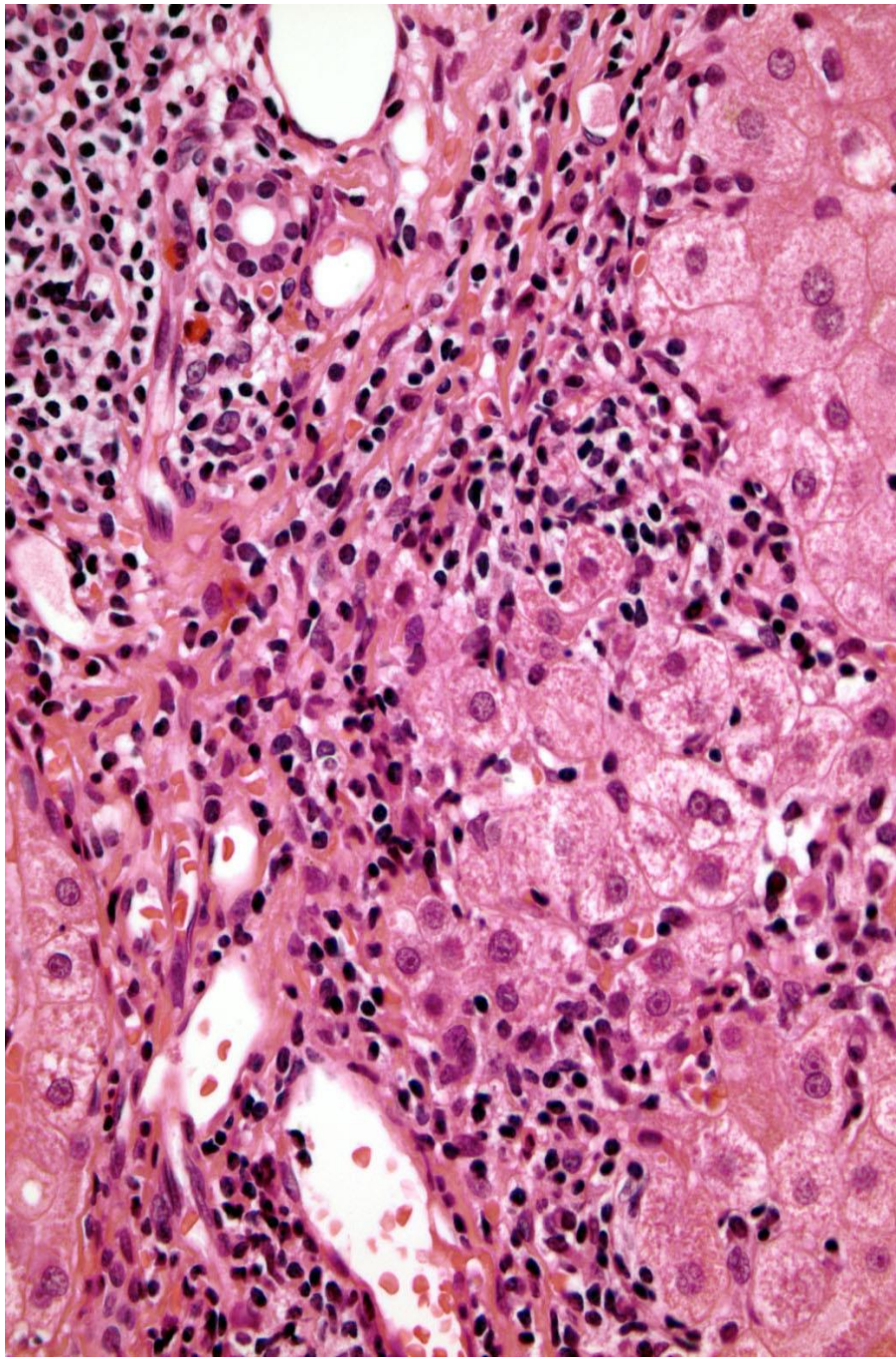
	Scores
Portal necrosis with or without bridging	0 - 10
Intralobular degeneration and necrosis	0 - 4
Portal inflammation	0 - 4
Fibrosis	0 - 4

(Knodell et al., Hepatology 1:431, 1981)

CDC


Chronic hepatitis C, mild form („chronic persistent hepatitis”)


Interface hepatitis



Modified HAI grading

necroinflammatory changes

		score
Grade A :	Interface hepatitis (piecemeal necrosis)	0–4
Grade B :	Confluent necrosis	0–6
Grade C :	Focal lobular activity	0–4
Grade D :	Portal inflammation	0–4
		maximum: 18

+ additional features
+ immunohistochemical findings

Ishak et al.: J Hepatol. 22 : 696-699, 1995

Staging of Chronic Hepatitis (×)

Score[1]	Description[2,3]	
0	no fibrosis	no fibrosis
1	mild fibrosis	extended fibrotic portal tracts
2	moderate fibrosis	periportal or portal - portal septa (intact architecture)
3	severe fibrosis	porto - central septa (architectural distortion)
4	cirrhosis	cirrhosis

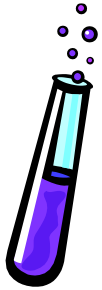
based on: [1] Knodell et al., 1981, [2] Scheuer 1991, [3] Sciot and Desmet 1994

CDC

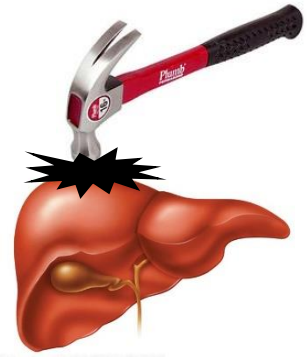
A májfibrosis mértékének megállapítása

- Májbiopszia : „the gold standard”

- Serum markerek

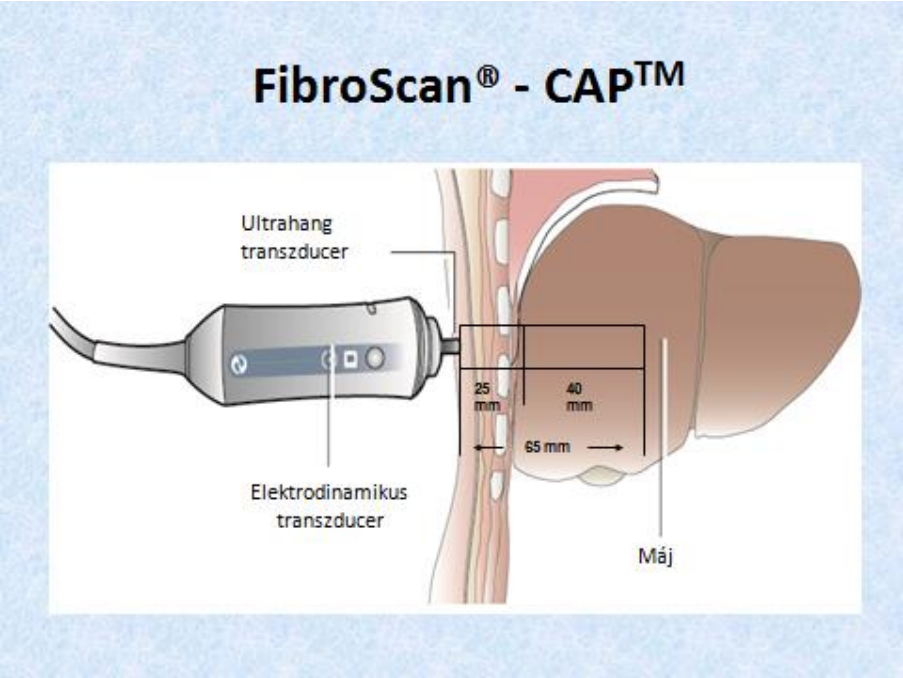


- Képalkotók : US, Fibroscan
  - („keménység”)



Fibroscan—CAP mérésének bemutatása

kb. 1 cm átmérőjű, 4 cm hosszú virtuális szövethengert mérünk, a bőrfelszíntől mért 25 és 65 mm távolság között.



Halász Tünde, PhD értekezés

Definition of AIH* (**)

AIH is an unresolving inflammation of the liver with:

- characteristic (but not specific) histology
- hypergammaglobulinaemia
- autoantibodies in the serum
- absence of „known” etiology (genetic, toxic, viral)

* Czaja A.J. Hepatology. A textbook of Liver Disease 2003.1163-1202, Saunders

Diagnostic criteria of AIH(1) * (**)

- Clinical: symptomatic (fatigue, fluctuating jaundice, arthralgia, acute/fulminant? etc.)
  asymptomatic
- Sex/Age: female predominance
  young (bimodal, children)
- Other: association with other autoimmune disorders

* Intern.Autoimmune Hepatitis Group
  J.Hepatol.1999.31:929-938

Diagnostic criteria of AIH (3)

Serological features*

Autoantibodies (Ab)

- Non-organ specific Abs
  - anti- nuclear AB (ANA)
  - anti- smooth muscle AB (SMA)
  - anti- neutrophilic cytoplasmic (pANCA)
- Liver-related Abs
  - asialoglycoprotein receptor (anti-ASGPR)
  - soluble liver antigen (anti-SLA) (liver-pancreas antigen - anti-LP)
  - liver cytosol type 1 (anti-LC1)
  - liver/kidney microsome type1 (anti-LKM1)

* Intern.Autoimmune Hepatitis Group J.Hepatol.1999.31:929-938

Classification of AIH

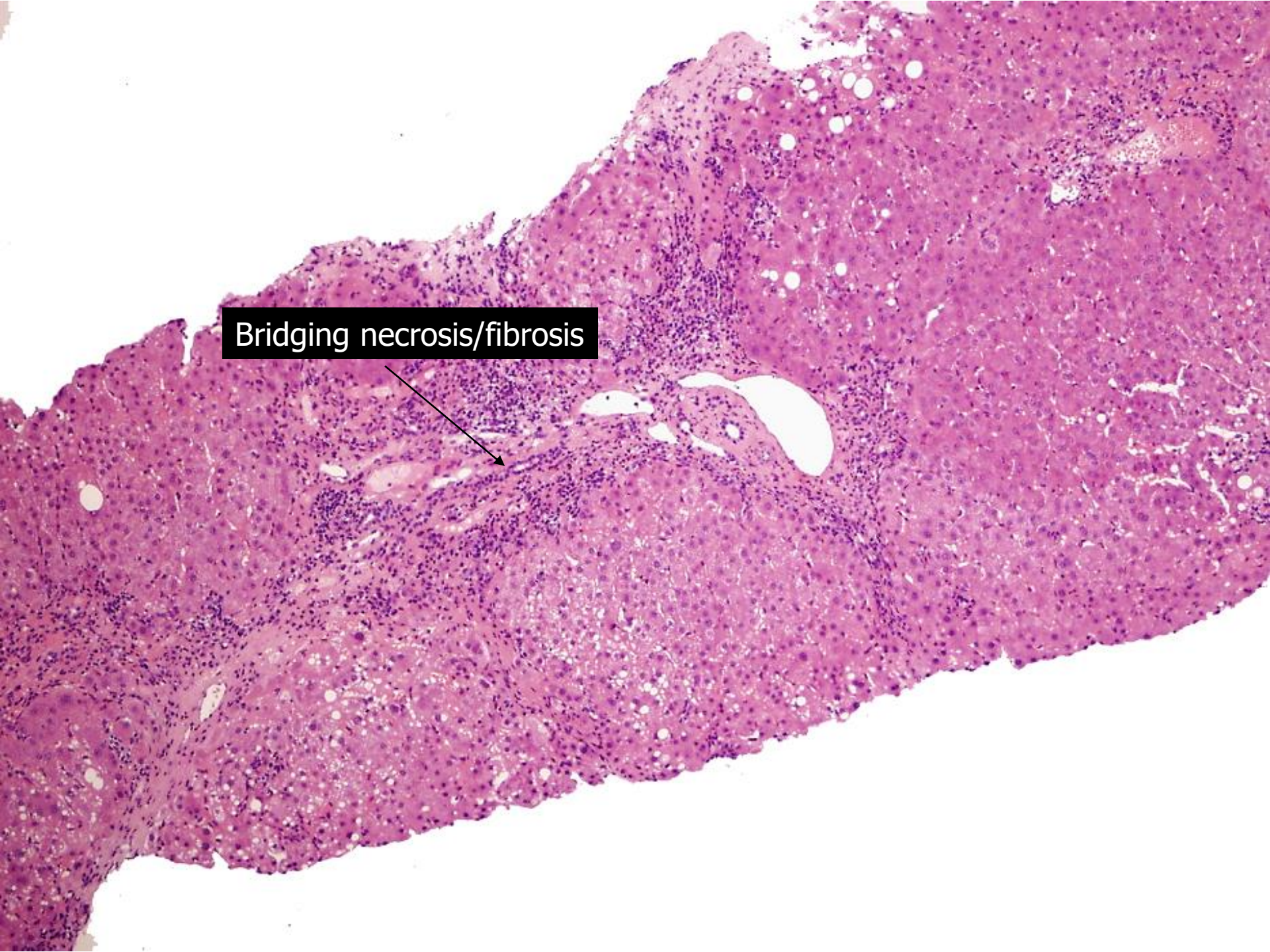
based on serological markers (antibody profiles)*

- Type 1: ANA/SMA positive (anti-SLA/LP positive, anti-ASSGPR, pANCA positive, 80%)
- Type 2: anti-LKM1 positive (almost always young females with severe disease, 3-4 %)
- Type 3: anti-SLA/LP positive (ANA/SMA positive, 3-4 %)

*** Czaja A.J., Manns M.P.: Am.J.Gastroenterol. 1995.90:1206.**

Diagnostic criteria of AIH (4) Histological features

- Interface hepatitis
  - periportal, periseptal
  - predominantly lymphoplasmocytic inflammation
- Mild/moderate acinar involvement
- Bridging necrosis/fibrosis
  - portal/portal
  - central/portal
- Rozette formation of hepatocytes
- Nodular regeneration
- No bile duct damage or granulomas
- Multinucleated **giant cells (children -23%)**
- **Cirrhosis**


Bridging necrosis/fibrosis