# Pathological Assessments in HCC

Stefan Hübscher,
School of Cancer Sciences, University of Birmingham
Dept of Cellular Pathology, Queen Elizabeth Hospital, Birmingham

# Pathological Assessments in HCC

1. Early HCC in cirrhotic liver
2. Well-differentiated HCC in non-cirrhotic liver
3. Role of pathology in prognosis and treatment

# Pathological Assessments in HCC

1. Early HCC in cirrhotic liver
2. Well-differentiated HCC in non-cirrhotic liver
3. Role of pathology in prognosis and treatment

Diagnosis of HCC arising in Cirrhotic Liver - Guidelines for the Use of Liver Biopsy

AASLD – Bruix & Sherman, Hepatology 2011: EASL-EORTC, J Hepatol 2012

**Liver biopsy not required for routine diagnosis of HCC (in cirrhotic livers )**

1. Radiological investigations have high diagnostic accuracy
   - Especially for lesions > 2cm diameter
   - Biopsy still recommended for smaller lesions (1-2cm), with inconclusive imaging

2. Risk of needle track seeding
   - Prevalence 1-2% (0-12.5%)
   - Higher frequency (50-500x) than with other abdominal tumours

**BUT**

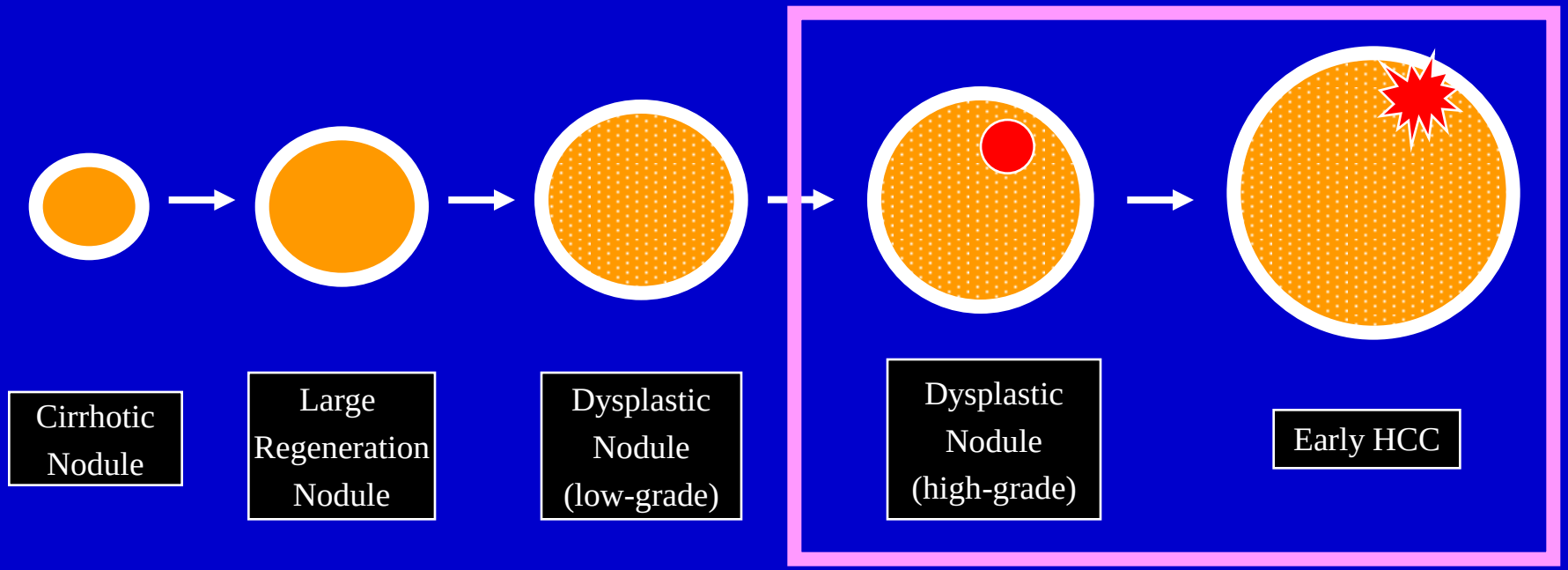
- Risks of seeding need to weighed against hazards of under- or over-diagnosing malignancy using non-invasive techniques
  - 16-31% of cases diagnosed as HCC prior to liver transplantation had alternative histological diagnoses (Libbrecht 2002, Baccarani 2006, Compagnon 2008)

- Meta-analysis of 1340 patients (8 studies) showed no impact of needle tract seeding on patient survival (Silva, Gut 2008; 57: 1592-1596)

# Evolution of HCC in Cirrhosis - Hepatocellular Carcinogenesis

- Multi-step process
- Successive stages associated with increase in: size of nodules
  molecular abnormalities
  risk of progression to HCC
- Overlapping morphological spectrum

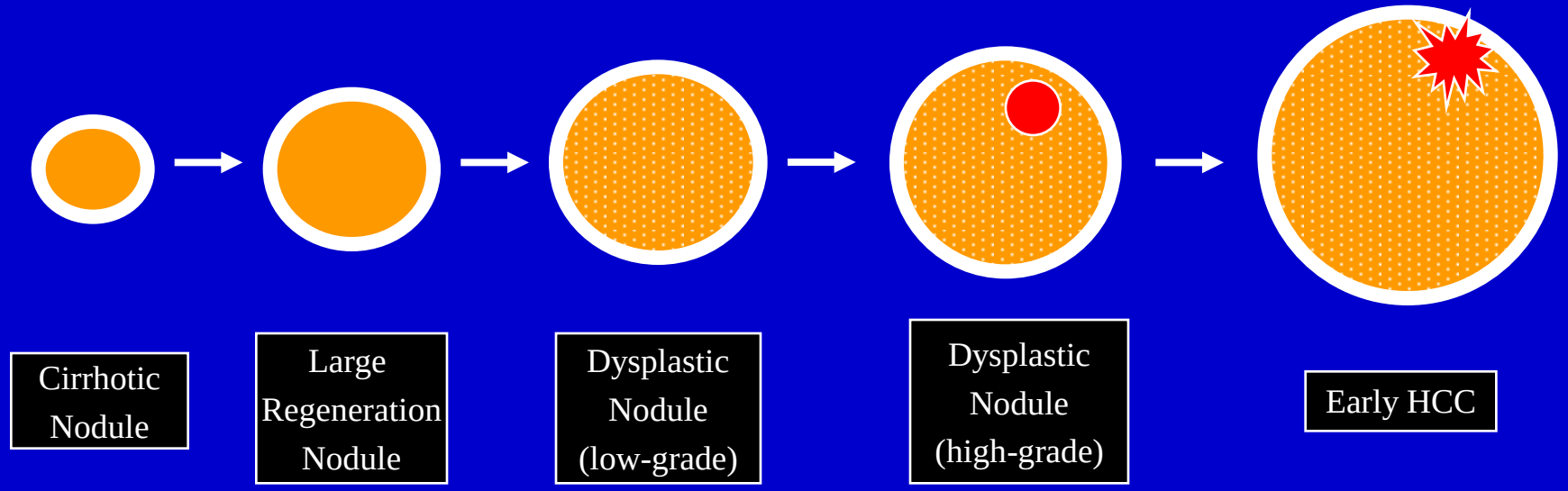
→ Increasing consensus regarding diagnostic criteria
(International Working Group- Hepatology 2009, WHO Classification 2010)



# Evolution of HCC in Cirrhosis

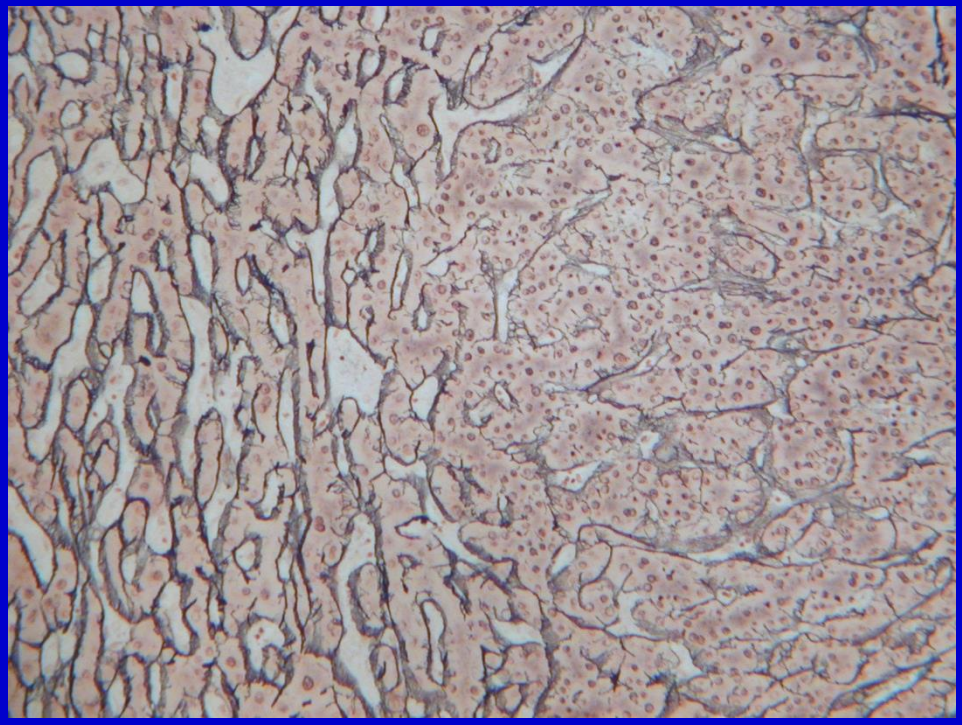
**Hepatocellular Carcinoma (early / well-differentiated) – Histological Features**

- Increased N/C ratio, nuclear crowding
- Cell plates > 3 cells thick, loss of reticulin framework
- Invasive growth (usually focal)
  - Stromal invasion
  - Capsular invasion
  - Vascular invasion (satellite nodules in surrounding liver)



Well-differentiated HCC arising in a dysplastic/cirrhotic nodule (Reticulin)

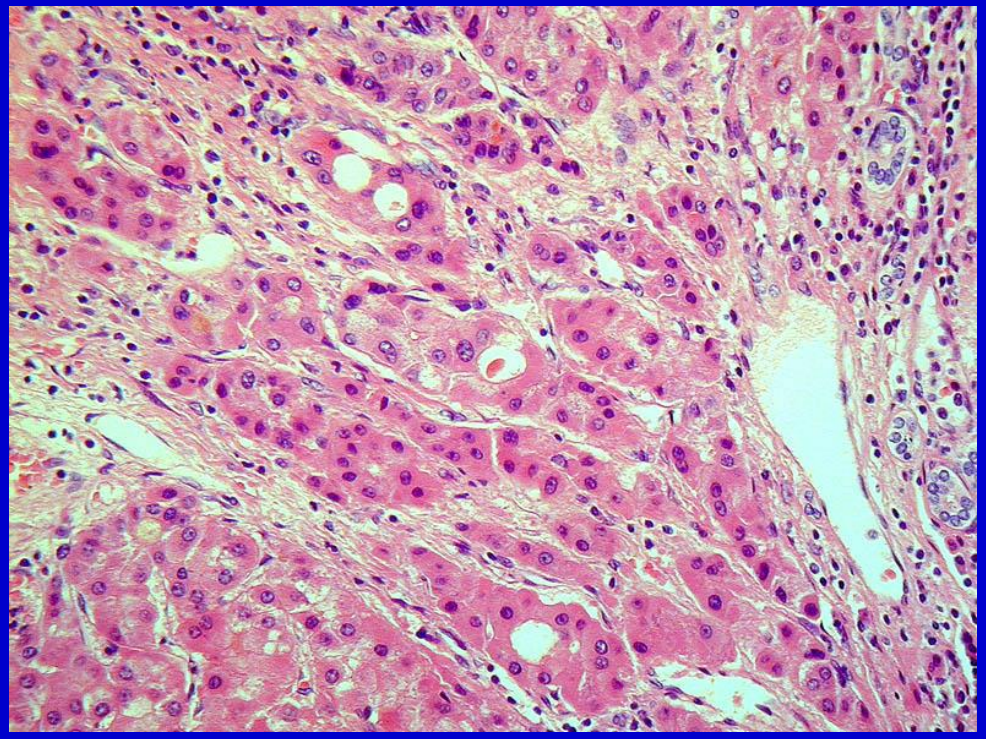
- "Nodule-in-nodule" growth pattern
- Nuclear crowding
- Thick cell plates
- Reticulin fibre depletion



**Problems with Reticulin Staining in HCC**

- Reticulin fibres may be preserved in well-differentiated/early HCC
- Focal reticulin fibre loss may occur in benign liver disease
  - e.g. Fatty liver disease (Singhi 2012)
  - Steatotic hepatocellular adenoma

# Patterns of Invasion in HCC

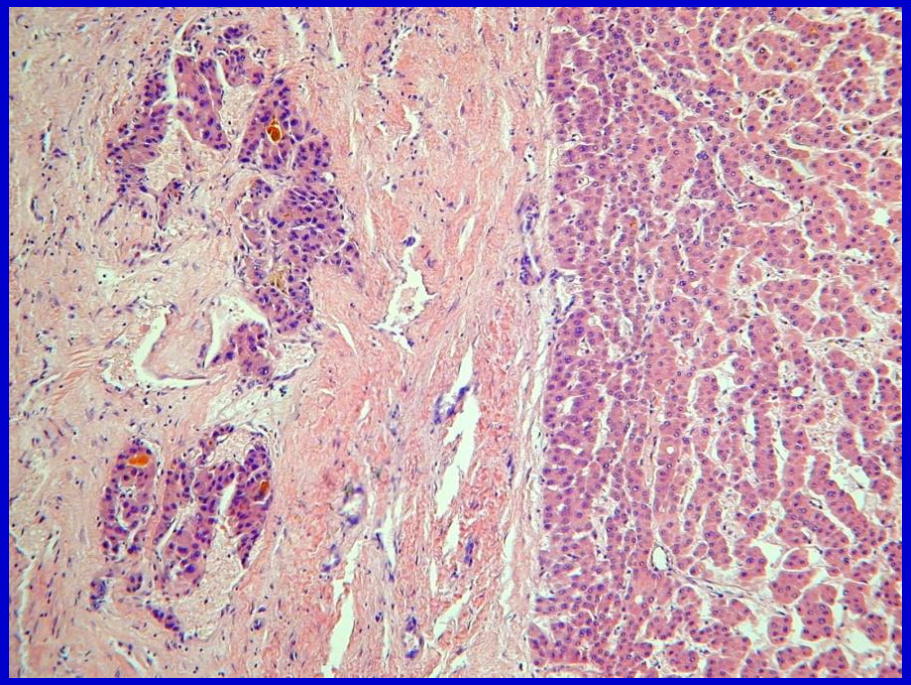


## Stromal Invasion

- Invasion of portal tract stroma within or outside lesion
- May be difficult to distinguish from entrapped non-neoplastic hepatocytes

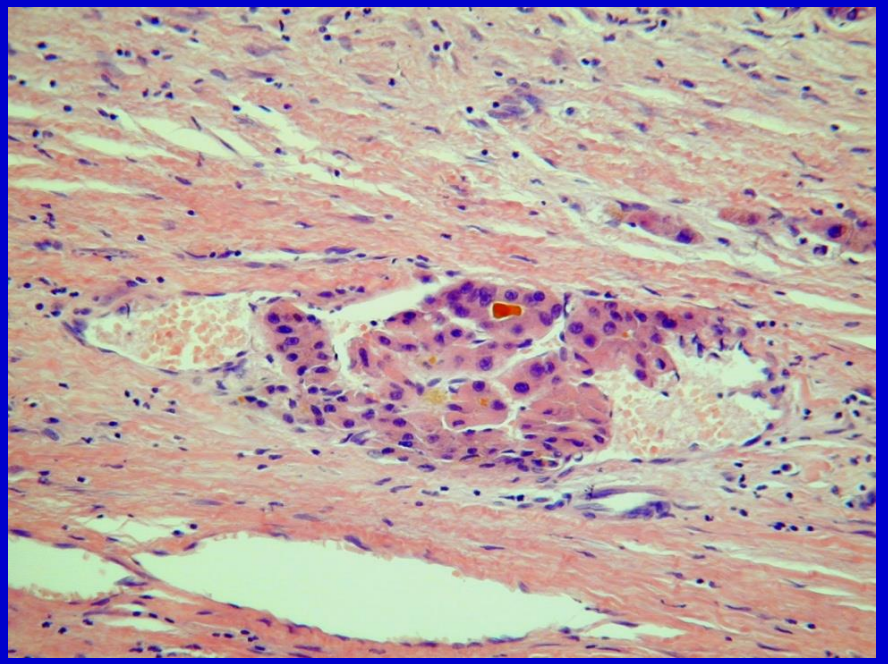
  → lack of ductular reaction ( shown by K7/K19 immunostaining) in neoplastic invasion (Park 2007, Bioulac-Sage 2011)

# Patterns of Invasion in HCC



**Capsular Invasion**

- Assessment similar to stromal invasion

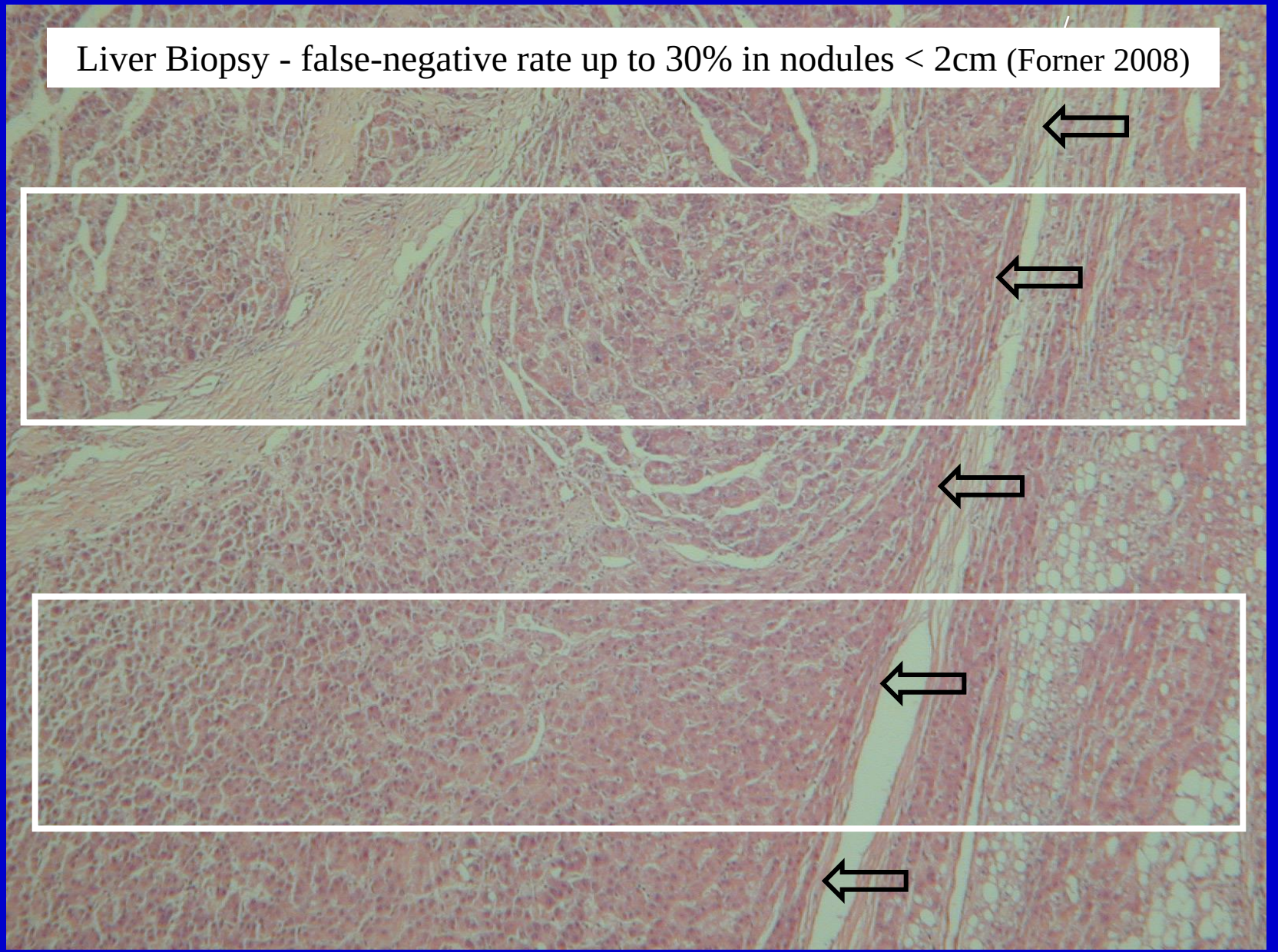


**Vascular Invasion**

- No longer “early” HCC

# NASH- associated cirrhosis

## 2cm diameter nodule with “nodule-in-nodule” growth pattern



# Benign Versus Malignant Nodules in Cirrhotic Livers
## Features Favouring a Diagnosis of Malignancy
## "Conventional" Immunohistochemical Markers

| **Antibody** | **Comment** |
|---|---|
| Ki 67 | Stepwise increase ( cirrhotic nodule – dysplastic nodule – early HCC)<br>No defined threshold for diagnosing HCC<br>Rarely exceeds 5% in well-differentiated HCC |
| SMA | Demonstrates/confirms arterialisation (also seen in HGDN) |
| CD34 | Diffuse capillarisation of sinusoids (vs focal in cirrhotic nodules)<br>May also occur in HGDN (and in FNH & liver cell adenoma) |
| AFP | Rarely positive in early HCC |

# Benign Versus Malignant Nodules in Cirrhotic Livers
## Features Favouring a Diagnosis of Malignancy - "Novel" Immunohistochemical Markers

Molecular studies have identified many genes up-regulated in early HCC

Some have products can be demonstrated immunohistochemically:

| **Genes/Proteins Upregulated in Early HCC** | | **Immunohistochemistry** (criteria for positivity) |
|---|---|---|
| **Glypican-3** | Heparan sulphate proteoglycan<br>Promotes growth of HCC by stimulating Wnt signalling | Cytoplasmic/membranous<br>(> 5-10% of cells) |
| **HSP 70** | Chaperone stress protein<br>Potent anti-apoptotic survival factor | Cytoplasmic/nuclear<br>(> 5-10% of cells) |
| **Glutamine Synthetase** | Target gene for beta- catenin,<br>GS overexpressed with activation/ mutation of beta-catenin,<br>Involved with hepatocyte regeneration/proliferation | Cytoplasmic<br>(diffuse >50%, unrelated to vessels) |

**Other markers upregulated in early HCC**

Clathrin heavy chain

Cyclase –associated protein 2 (CAP2)

Des-gamma-carboxyprothrombin (DCP)

EZH2

LYVE-1

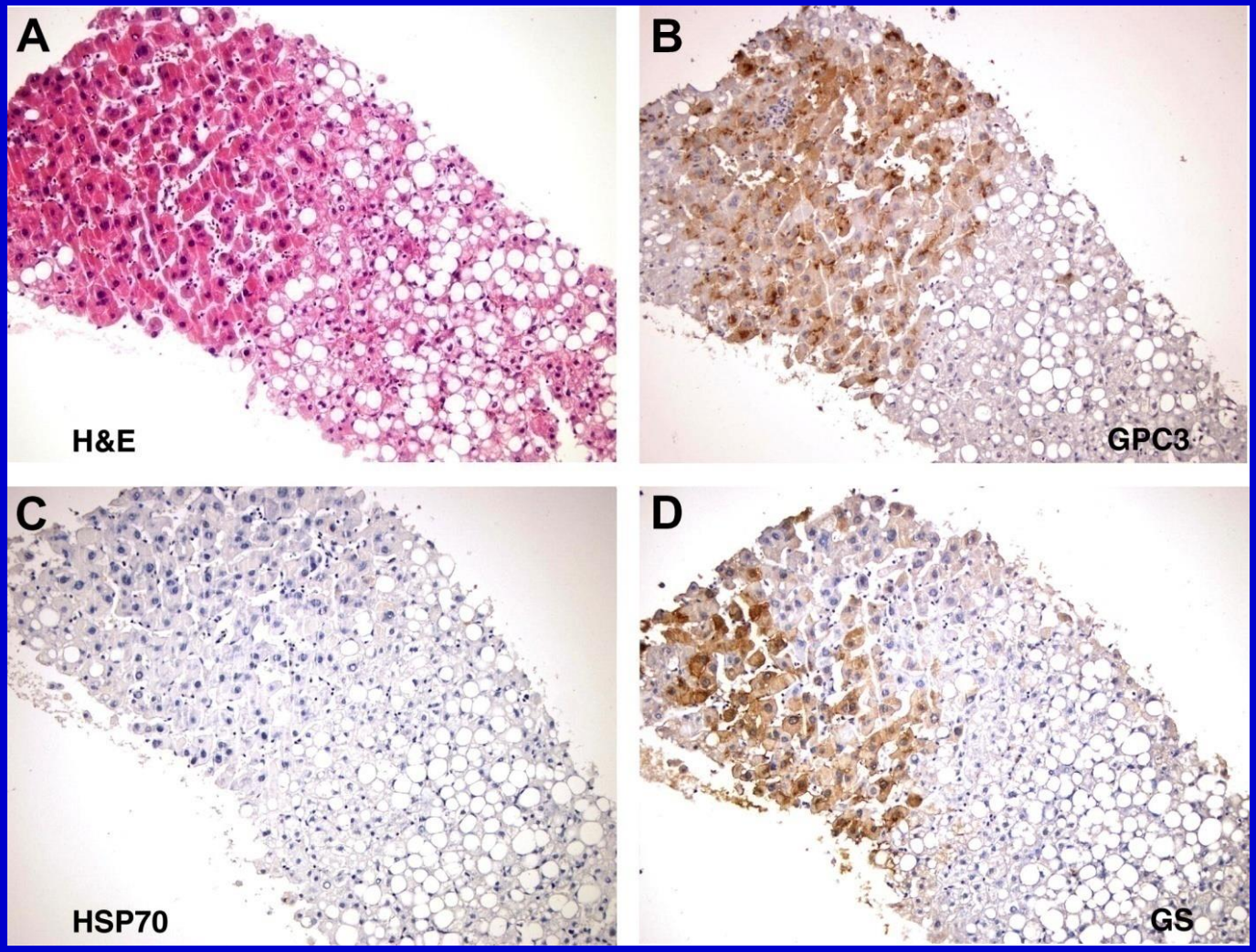
Survivin

# Benign Versus Malignant Nodules in Cirrhotic Livers
## Features Favouring a Diagnosis of Malignancy - "Novel" Immunohistochemical Markers

**Application (**Glypican-3, HSP 70, Glutamine Synthetase)

- None has 100% specificity or sensitivity individually
- Panel of antibodies improves diagnostic accuracy
  - ≥ 2/3 positive - 100% specificity & 60-70 % sensitivity for HCC
    (Di Tommaso 2009, Tremosini 2012)
  - Addition of clathrin heavy chain to panel improves sensitivity for small HCC
    (Di Tommaso 2011)

# Immunohistochenmistry to Diagnose Early HCC in Liver Biopsy Specimens
(from Di Tommaso 2009)



# Immunohistochemistry to Diagnose Early HCC
# Problems & Limitations
(Sherman , Hepatology 2011)

1. Reproducibility of staining methods

2. Conventional histology remains the "gold standard"

- Studies investigating new antibodies use routine histological assessments to define dysplastic nodules and early/well-differentiated HCC

- Recent prospective study concludes that use of an immunohistochemical panel (GPC-3/HSP 70/ GS) "only slightly increases diagnostic accuracy over expert morphological analysis" (Tremosini, Gut 2012)
  - Diagnosis changed in 1/40 cases (from dysplastic nodule to HCC ) eventually confirmed as HCC

- Immunohistochemical panel should only be used as an adjunct to conventional histological assessment

# Pathological Assessments in HCC

1. Early HCC in cirrhotic liver
2. Well-differentiated HCC in non-cirrhotic liver
3. Role of pathology in prognosis and treatment

# Well-differentiated HCC in non-cirrhotic liver

- Majority have risk factors for chronic liver disease and show pre-cirrhotic fibrosis
  - Increasing recognition of HCC arising in background of pre-cirrhotic NAFLD ( 40 - 65% - Paradis 2009, Yasui 2011, Duan 2012)
- Occasional cases arise in apparently normal livers with no recognised risk factors for liver disease (may be genetically distinct –Tretiakova 2010)
- Radiological criteria for diagnosing malignancy not reliable in this setting (EASL-EORTC Guidelines 2012, Forner 2012)

# Hepatocellular Carcinoma – Differential Diagnosis
## Well-differentiated HCC (in non-cirrhotic liver)

**Benign Hepatocellular Lesions**

- Focal nodular hyperplasia
- Hepatocellular adenoma

**Malignant hepatocellular neoplasms**

- Hepatoblastoma (macrotrabecular type)
- Fibrolamellar carcinoma

# Hepatocellular Adenoma (HCA) and Well-Differentiated HCC

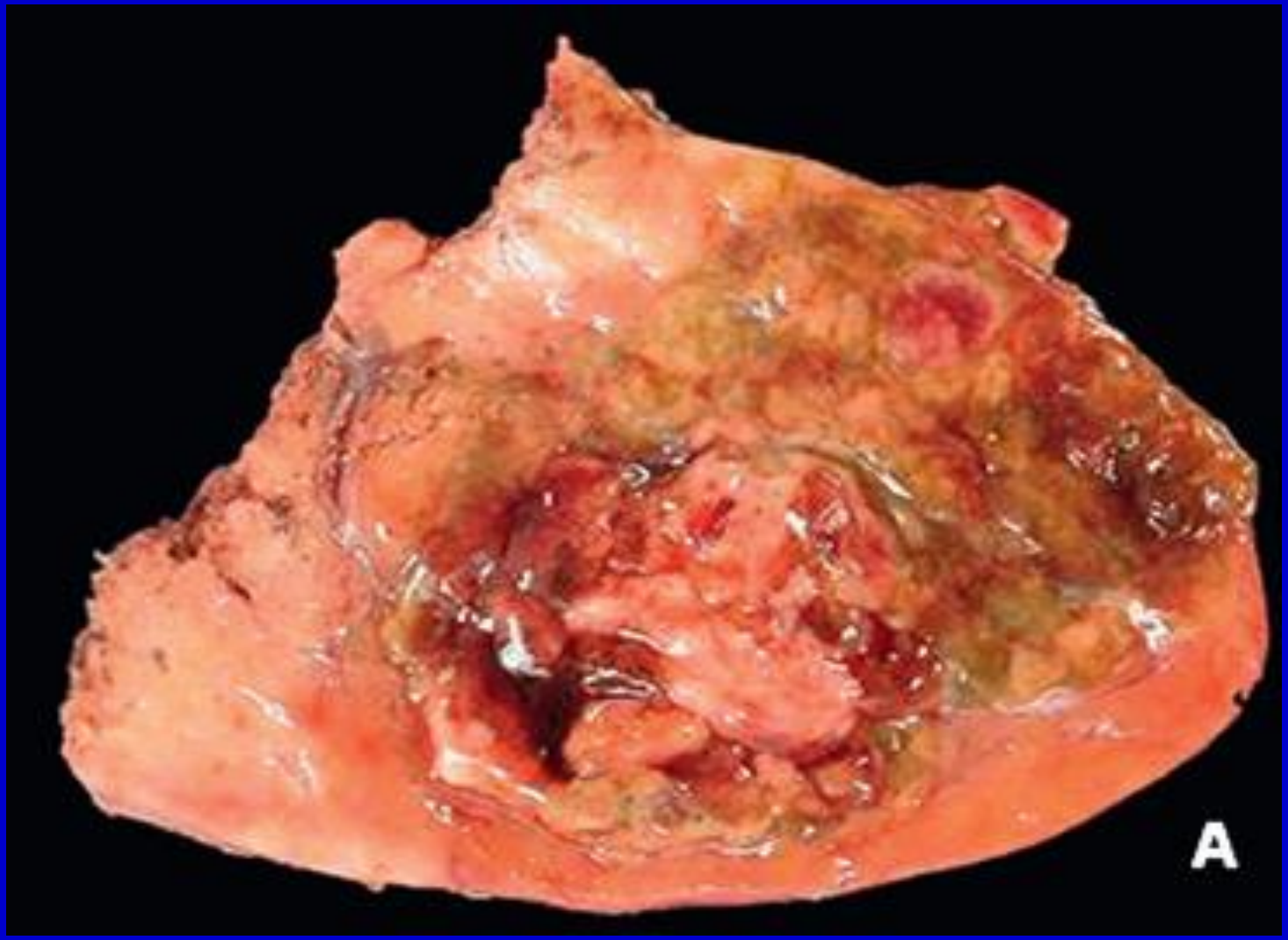
Histological distinction between HCA and well-differentiated HCC can be very difficult, particularly in needle biopsy specimens

HCA predominantly occurs in women, age 15-45, with no evidence of chronic liver disease

Malignant potential of HCA :

- Malignant transformation occurred in 68/1635 (4.2%) published cases of hepatocellular adenoma (Stoot 2010)
- >90% occur in lesions > 5cm diameter ( Stoot 2010, Farges 2011, Bellamy 2012)
- Higher risk of malignant transformation in men (47%) than women (4%) (Farges 2011)

# HCC Arising in Liver Cell Adenoma
(From Farges, Gut 2011)




1 cm nodule with features of HCC within 3cm liver cell adenoma

# Does Non-Cirrhotic HCC Arise From Hepatocellular Adenoma?

**NO**

- 52 HCCs resected from non-cirrhotic liver (Witjes 2012)
  - no evidence for adenoma component

POSSIBLY

- 22/74 (30%) cases of non-cirrhotic HCC had features suggesting possible derivation from adenoma (Liu, Mod Pathol 2013)

# Hepatocellular Adenoma
## Recent Developments in Genotypic and Phenotypic Classification
(Bioulac Sage 2011 & 2012 )

| **Adenoma Subtype** | **Frequency** | **Molecular Alterations** | **Immuno-phenotype** | **Clinico-pathological features** | **Malignant potential** |
|---|---|---|---|---|---|
| HNF1α Inactivated (H-HCA) | 30-35% | Hepatocyte nuclear factor 1 α inactivation | Absent staining for liver fatty acid binding protein (LFABP) | Marked steatosis (due to lack of LFABP) | Very low |
| β–catenin activated (b-HCA) | 5-10% | β–catenin activation | Nuclear β–catenin<br>Glutamine synthetase | More common in men<br>Cytological atypia, pseudoglandular formation | Up to 40% |
| Inflammatory (IHCA) | 50-60% | IL-6 /STAT3 activation | Serum amyloid A<br>C-reactive protein | Sinusoidal dilation (“telangiectatic”) & abnormal vessels<br>Inflammatory infiltrates<br>Ductular reaction (FNH-like)<br>Associated with metabolic syndrome & steatosis in background liver<br>β–catenin mutation in up to 10% | Low (increased in male patients, obesity , β–catenin activation) |
| Unclassified | < 10% | | | | |

Immunophenotypic characterisation also possible in biopsy specimens

Overall 15-20% of HCA are β– catenin activated

More frequent in men, associated with cytological atypia, malignant transformation in 30-40%

# Beta-catenin Mutated Liver Cell Adenoma

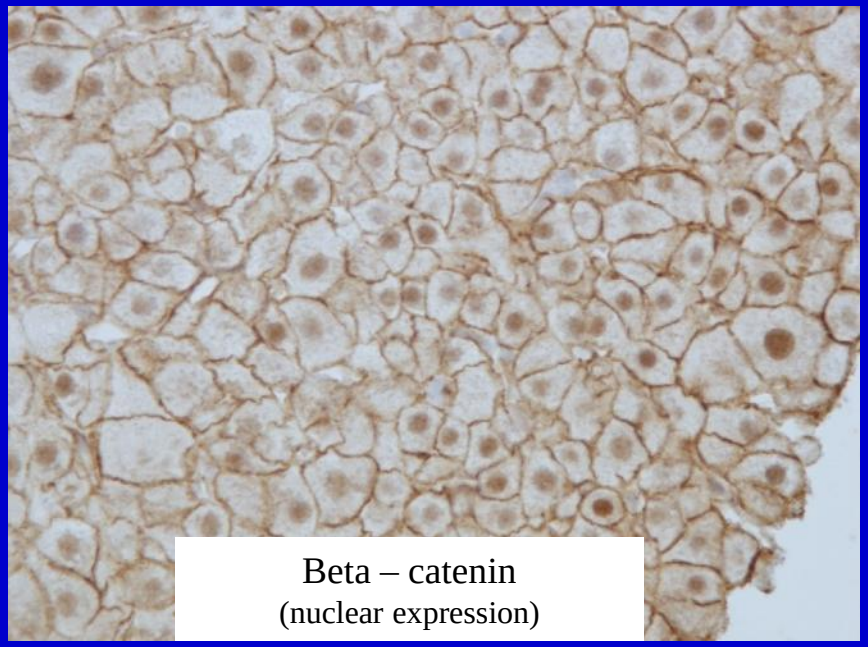
- **Molecular studies (qRT-PCR)** (Zucman-Rossi 2006)
  - Transcriptional activation of β -catenin targeted genes - GS, GPR49

- **Immunohistochemistry** (Bioulac-Sage 2007 & others)
  1. Nuclear (+/- cytoplasmic) expression of β– catenin
  2. Diffuse staining for glutamine synthetase

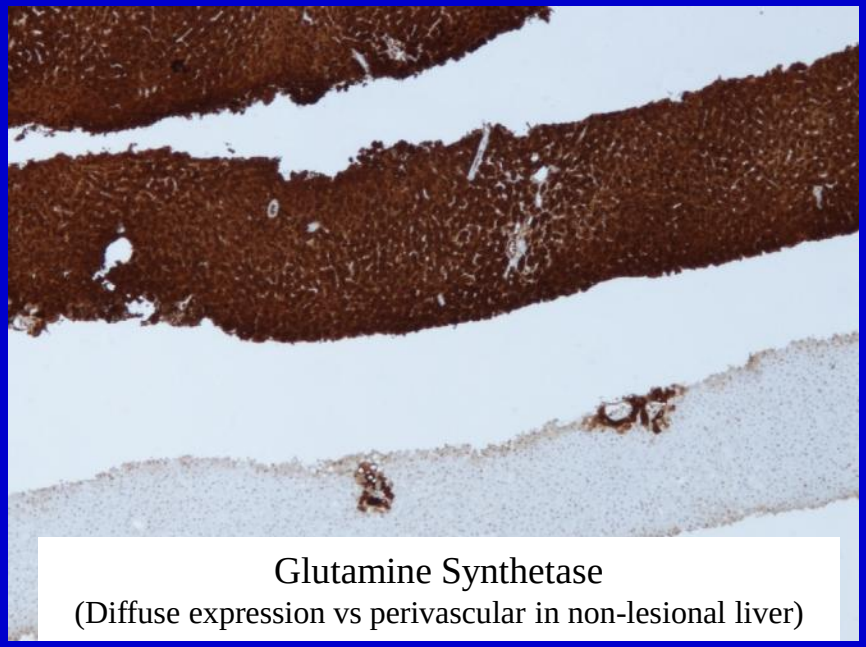
  B-cat 100% specific, 85% sensitive. GS 89% specific, 100% sensitive

- Combination of 1 & 2 reliable for identifying β– catenin activated lesions
- Problematic cases (e.g. diffuse GS staining without nuclear β– catenin ) still require molecular studies for confirmation

# Beta-Catenin Mutated Liver Cell Neoplasm



Beta – catenin
(nuclear expression)



Glutamine Synthetase
(Diffuse expression vs perivascular in non-lesional liver)

**Male, age 31** (Gnomes Meeting – Birmingham Case A/2013)

- 11 cm mass in right lobe of liver ( not resectable)
- Well differentiated hepatocellular lesion with no cytological atypia
- Presence of focal reticulin fibre loss, glypican 3 positivity, diffuse sinusoidal CD34 expression suggestive of well-differentiated HCC
- Some atypical hepatocellular neoplasms may be difficult to classify
  ("Atypical hepatocellular adenoma - like neoplasms" - Evason 2013)

# Pathological Assessments in HCC

1. Early HCC in cirrhotic liver
2. Well-differentiated HCC in non-cirrhotic liver
3. Role of pathology in prognosis and treatment
    - Conventional histological grading & staging
    - Other markers relevant for prognosis (and treatment)

# Hepatocellular Carcinoma – Histological Grading

- Edmonson & Steiner (1954) - 4 grades. Still widely cited

- More recent studies advocate use of 3-point grading system (Pomfret 2010)
  - Well-differentiated (equivalent of Edmonson & Steiner grades 1 and 2)
  - Moderately-differentiated (Edmonson & Steiner grade 3)
  - Poorly –differentiated (Edmonson & Steiner grade 4)

**Clinical Relevance:**

1. Tumour grade in resected specimens independently prognostic of outcome following local resection and liver transplantation

2. Grading in liver biopsies may be subject to tumour heterogeneity

**BUT**

- Grade in pre-operative biopsy (well/moderate vs poorly-differentiated) correlated with final grade in 74/81 (91% ) patients undergoing resection for HCC (Colecchia 2011)
- Grade in pre-operative biopsy accurately predicted survival

# Hepatocellular Carcinoma – Pathological Staging
# UICC –TNM Classification, 7th Edition, 2009

| | |
|---|---|
| pT0 | No evidence of primary tumour |
| pT1 | Solitary tumour without vascular invasion |
| pT2 | Solitary tumour with vascular invasion or multiple tumours , none more than 5 cm in greatest dimension |
| pT3a | Multiple tumours, any more than 5 cm |
| pT3b | Single or multiple tumours of any size involving a major branch of the portal vein or hepatic vein |
| pT4 | Tumour(s) with direct invasion of adjacent organs other than the gall bladder or with perforation of visceral peritoneum. |

- Pathological assessments of resection specimens /hepatectomy specimens at liver transplantion tend to up-stage pre-operative radiological assessments:
  - microscopic vascular invasion
  - small nodules (<1cm), including satellite nodules
- Clinical significance of pathological upstaging uncertain

# Pathological Assessments in HCC

1. Early HCC in cirrhotic liver
2. Well-differentiated HCC in non-cirrhotic liver
3. Role of pathology in prognosis and treatment
    - Conventional histological grading & staging
    - Other markers relevant for prognosis (and treatment)

# Other Prognostic/Therapeutic Markers in HCC

**Techniques Used**

- Global gene expression profiling
  - DNA microarrays
- Specific candidate proteins/ genes
  - Immunohistochemistry, RT-PCR

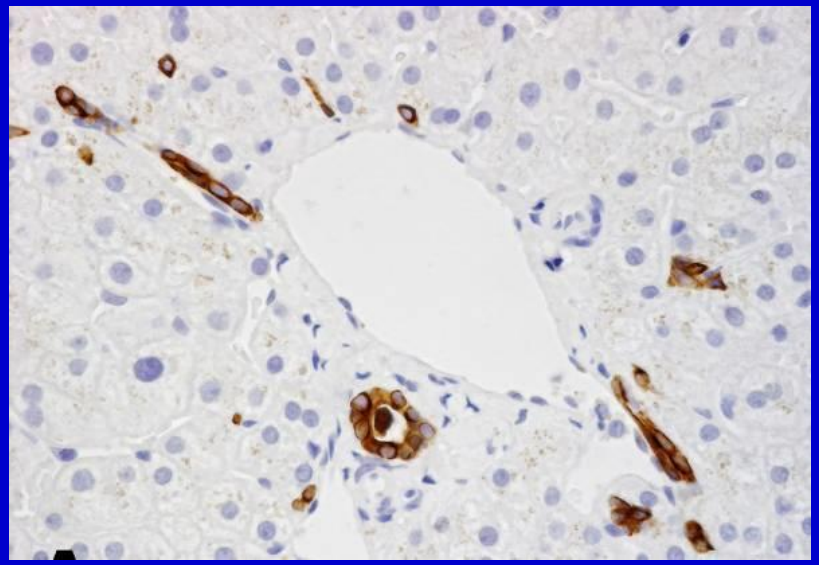
**Possible applications for therapy**

- Some molecular alterations common to most HCCs
  - e.g. Apoptosis , cell proliferation , angiogenesis
- Others more restricted
  - e.g. Wnt activation, mTOR signalling, progenitor cell phenotype
  - Increased use of liver biopsy (for targeted therapy)

# Progenitor Cell Phenotype in HCC

- Tissue markers of hepatic progenitor cells
  - K 19, CD 133, EpCAM, c-kit, OV6, sox-9

- Immunostaining for K 19 as a marker of a progenitor cell phenotype
  - Embryonic liver has bipotential K19+ progenitor cells (hepatoblasts) which can differentiate into hepatocytes and bile ducts
  - In normal adult liver K 19 expression confined to biliary epithelial cells and putative progenitor cells in canals of Hering



# Progenitor Cell Phenotype in HCC

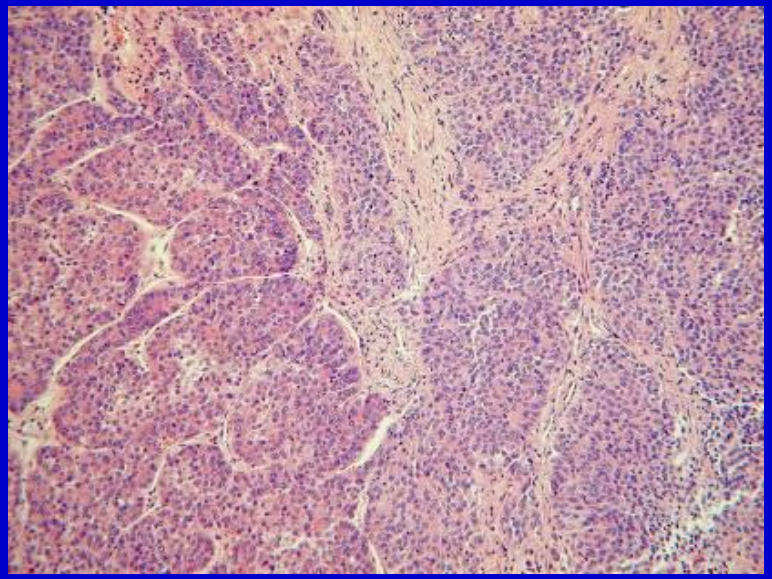
- 10-20% of HCCs express K19 (> 5% cells positive)
  - worse prognosis than K19-negative HCCs
  - K19 positivity in biopsies with HCC also strongly predictive (van Malenstein 2012, Govaere 2013)

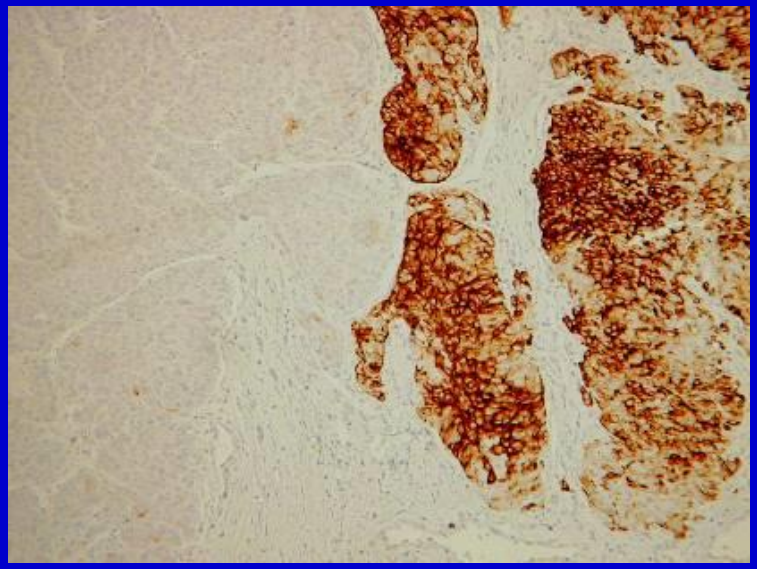
- K 19 positive cells may have an intermediate (progenitor cell-like) morphology
- Some may also show glandular differentiation (resembling cholangiocarcinoma) and express other biliary markers

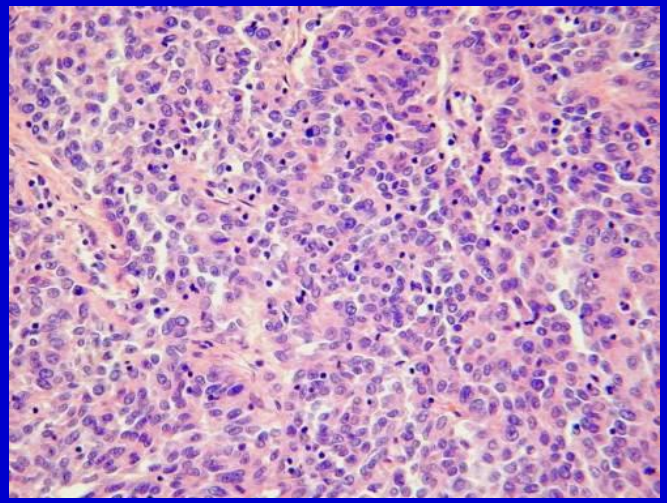
- More frequent expression of EMT-related proteins and factors associated with angiogenesis and invasion/metastasis (Kim 2011, Yang 2012, Govaere 2013)
- Longer telomeres (Kim 2013)
  - Increased expression of telomerase (hTERT) and shelterins (TRF2, TPP1)

# K 19 Immunostaining in HCC

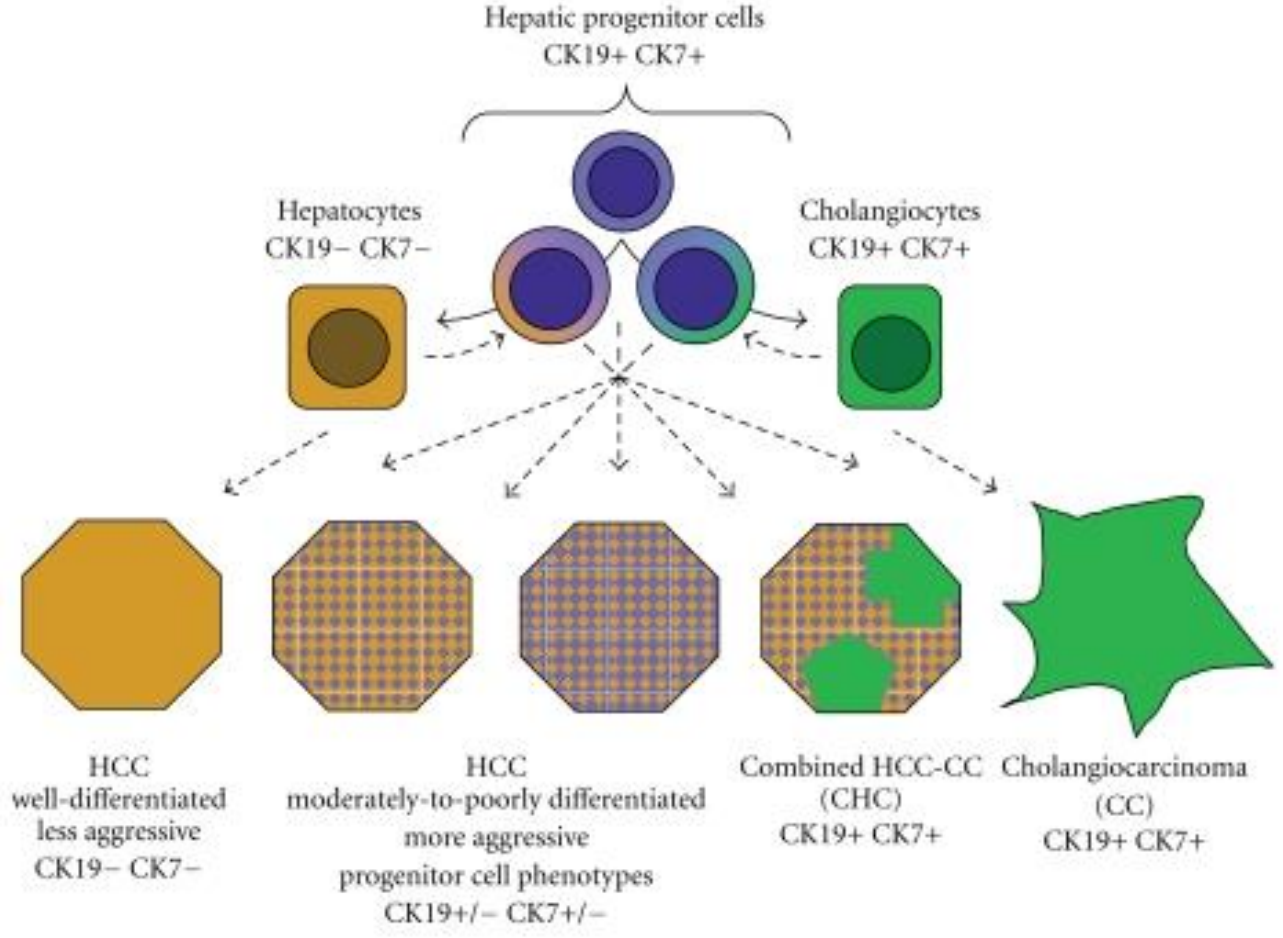
- Female, age 43. Cirrhosis complicating acute seronegative hepatitis 20 years ago
- 5 cm diameter mass in right lobe (MRI = HCC)







# Progenitor Cell Origin of Malignant Epithelial Neoplasms in the Liver
## Morphological Continuum between HCC and Intrahepatic Cholangiocarcinoma (peripheral mass –forming type)




Walther and Jain (2012)

# Progenitor Cell Phenotype in HCC

**Therapeutic implications**

- K19+ tumour cells resistant to chemotherapy - doxorubicin, 5-fluorouracil and sorafenib (Govaere 2013)
  - Increased expression of multi-drug resistance proteins – e.g. ABC-G2, MRP-1 (Van der Borght 2008, Oikawa 2013, Sun 2013)

- Potential application for molecular targeted therapy
  - K19 positivity/ progenitor cell phenotype associated with upregulation of:
    - CD13 (Marquardt 2012)
    - EGFR (Kim 2011)
    - EpCAM (Yamamshita 2010)
    - microRNAs - e .g. MiR-141, miR-200 (Govaere 2013)
    - Wnt/B-catenin (Dahmani 2011, Govaere 2013)
    - PDGFRA (Govaere 2013)

And, finally…….

# Barclay's Premier League, Old Trafford, Saturday 28th September 2013


1. Name the player who has just scored, leading to an historic victory
2. What was the final score?
3. When did the team playing in blue & white stripes last win a league match at Old Trafford?

# Barclay's Premier League., Old Trafford, Saturday 28th September 2013


1. Name the player who has just scored, leading to an historic victory (1 mark)
2. What was the final score? (1 mark)
3. When did the team playing in blue & white stripes last win a league match at Old Trafford? (I mark)

1. Saido Berahino
2. Manchester United 1 – West Bromwich Albion 2
3. 1978

# Noncirrhotic hepatocellular carcinoma: derivation from hepatocellular adenoma? Clinicopathologic analysis

Ta-Chiang Liu[1], Neeta Vachharajani[2], William C Chapman[2] and Elizabeth M Brunt[1]

Modern Pathology 2013, in press

- 22/74 (30%) cases of non-cirrhotic HCC had features suggesting possible derivation from adenoma.

1. Morphological features compatible with HCA and HCC in same lesion (n=10)
2. Steatotic tumour lacking LFABP staining ( n=4)
3. Diffuse SAA expression and features compatible with inflammatory adenoma (n=4)
4. Nuclear/ctyoplasmic beta-catenin and diffuse glutamine synthetase ( n=4)