Case 1 - Clinical Summary

Male, age 56

- Liver resection for metastatic colorectal carcinoma.
- Incidental small subcapsular lesion 0.5 cm diameter in segment 6.
Case 1 – Histological Findings

- Small, slightly irregular glands
- No cytological atypia
- Scanty fibrous stroma containing inflammatory cells
- Incorporated portal tract

Case 1 – Diagnosis

Peribiliary gland hamartoma (bile duct adenoma)

Case 1 – Discussion Points

1. Nature of lesion (evidence for peribiliary gland origin)
   - Topographic relationship to normal bile duct
   - Immunohistochemical studies show phenotype of peribiliary glands rather than bile ducts (D10 & 1F6 positive) (Bharath 1996)
   - Similar changes may occur as reaction to localised area of parenchymal extinction (e.g. in cirrhotic livers)
     ➢ “peribiliary gland hyperplasia”
   - Minimal malignant potential

BUT
- Recent studies have shown BRAF V600E mutations in 55% of cases, suggesting bile duct adenoma is a neoplasm (Pujals Hepatology 2014 & Pujals Histopathology 2015)
- Similar mutations seen in 5% of intrahepatic cholangiocarcinomas

Case 1 – Discussion Points

2. Clinical Presentation
   - Incidental finding at abdominal surgery
   - Common indication for frozen section

Case 1 – Discussion Points

3. Features favouring a benign process
   - Small size
   - Lack of cytological atypia
   - Fibrous stroma dense & inflamed (but not desmoplastic)
     ➢ Some lesions may have abundant hyalinised fibrous stroma with relatively scanty glands
   - Incorporated portal tracts
Case 2

- Clinical Summary

Female, age 51
- Liver resection for three lesions in left lobe.
- Main lesion 8cm
  - histology = hepatocellular adenoma
- Slide submitted is from a 4.5cm lesion, which had an irregular central scar
- 3rd lesion 1.5cm diameter had similar histological appearances to lesion 2, but lacked a central scar
Glutamine Synthetase

- confined to narrow zone of perivenular hepatocytes in normal liver
- “map-like” pattern of over-expression in FNH
  - reflects beta-catenin activation without mutation
Case 2 – Histological Findings

- Well-differentiated hepatocellular lesion, showing no cytological atypia
- Central fibrous scar containing abnormal blood vessels
- Radiating fibrous septa containing arteries (without bile ducts), inflammatory cells and marginal zones of ductular reaction
- “Map-like” overexpression of glutamine synthetase

Case 2 – Diagnosis

Focal Nodular Hyperplasia

Case 2 – Discussion Points

1. Pathogenesis of FNH
   - Response to altered blood flow (increased arterial flow)
     - Hepatic or portal venous injury as primary event
     - Congestion and parenchymal collapse (fibrous septa)
     - Arterio-venous shunting and hyper-arterialisation (loss of portal veins and bile ducts)
   - May be primary or secondary

<table>
<thead>
<tr>
<th>Type</th>
<th>Pathogenesis / Histological Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>Primary vascular abnormality (congenital or acquired)</td>
</tr>
<tr>
<td></td>
<td>Central scar with abnormal blood vessels (x)</td>
</tr>
<tr>
<td>Secondary</td>
<td>FNH-like changes present in association with vascular abnormalities related to other liver diseases (focal or diffuse)</td>
</tr>
</tbody>
</table>
<pre><code>                 | Focal lesions – e.g. haemangiona, hepatocellular adenoma                  |
                 | Diffuse disease – e.g. Cirrhosis, Budd-Chiari syndrome                   |
                 | May have different features to primary FNH (Bocchini 2008)              |
</code></pre>

2. Problems with liver biopsy interpretation
   - Features of FNH can mimic other liver diseases
     - Cirrhosis
     - Chronic biliary disease with bile duct loss
     - Nearby space-occupying lesion

3. FNH versus Hepatocellular Adenoma

<table>
<thead>
<tr>
<th></th>
<th>FNH</th>
<th>HCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central Scar</td>
<td>Yes (contains abnormal blood vessels)</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Some FNH, especially small lesions, may lack clearly identifiable scar</td>
<td>Foci of fibrosis related to old haemorrhage/necrosis</td>
</tr>
<tr>
<td>Nodular Growth</td>
<td>Pattern</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Typical</td>
<td>Fibrous</td>
</tr>
<tr>
<td>Ductular Reaction</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes (includes fibrovascular septa, inflammatory cuffs often present)</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>No readily identifiable ductules (arterial branches +/- fibrous tissue present without ductules)</td>
<td>Inflammatory sub-epithelial foci of SB &amp; inflammation (previously classified as “telangiectatic FNH”)</td>
</tr>
<tr>
<td>Glutamine synthetase expression</td>
<td>“Map-like” distribution adjacent to hepatic veins</td>
<td>Mostly negative or perivascular diffusely positive in beta catenin mutated HCA</td>
</tr>
</tbody>
</table>
Case 3 - Clinical Summary

Female, age 37

- Left lobe resection for 10 cm mass bulging from surface.
- Slide submitted is from periphery of lesion to include adjacent non-neoplastic liver tissue.

Tumour Periphery (including surrounding non-lesional liver)

Reticulin
Serum Amyloid A (SAA)

- Tumour
- Non-lesional liver

Glutamine Synthetase

- Tumour
- Non-lesional liver
Case 3 – Histological Findings

- Well-differentiated hepatocellular lesion
- Fibrovascular structures containing foci of inflammation and ductular reaction
- Sinusoidal dilatation and peliosis
- No cytological atypia. Reticulin framework well preserved.

- Immunohistochemistry
  - Diffuse CRP and SAA expression favours inflammatory phenotype
  - No features to suggest beta-catenin mutation

- Uninvolved liver shows mild fatty change (probably NAFLD – BMI 33)

Case 3 – Diagnosis

- Hepatocellular adenoma (inflammatory subtype)
- No evidence of malignancy
- Arising in background of fatty liver (metabolic syndrome)

Hepatocellular Adenoma – Molecular Classification (Bioulac-Sage et al)

1. Molecular studies have sub-classified HCAs according to different gene mutations
2. Subsequent studies, using resection specimens, have shown that these HCA subtypes have different clinico-pathological features and immunostaining profiles
3. Immunophenotypic characterisation may also be possible in liver biopsy specimens
4. Findings may be relevant for clinical management – e.g. identifying lesions with an increased risk of malignant transformation
Liver Cell Adenoma
Recent Developments in Genotypic and Phenotypic Classification
(Bioulac Sage 2011)

<table>
<thead>
<tr>
<th>Adenoma Subtype</th>
<th>Frequency</th>
<th>Molecular Alterations</th>
<th>Immuno-phenotype</th>
<th>Clinico-pathological Features</th>
<th>Malignant potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>HNF1a Inactivated (H-HCA)</td>
<td>30-35%</td>
<td>Hepatocyte nuclear factor 1a inactivation</td>
<td>Absent staining for liver fatty acid binding protein (LFABP)</td>
<td>Marked steatosis (due to lack of LFABP)</td>
<td>Very low</td>
</tr>
<tr>
<td>b-catenin activated (b-HCA)</td>
<td>5-10%</td>
<td>b-catenin activation</td>
<td>Nuclear b-catenin expression</td>
<td>b-catenin mutation in up to 10%</td>
<td>Very low</td>
</tr>
<tr>
<td>Inflammatory (IHCA)</td>
<td>50-60%</td>
<td>IL-6/STAT3 activation</td>
<td>Serum amyloid AC-reactive protein</td>
<td>Sinusoidal dilation (&quot;telangiectatic&quot;) &amp; abnormal vessels</td>
<td>Very low</td>
</tr>
<tr>
<td>Unclassified</td>
<td>&lt;10%</td>
<td></td>
<td></td>
<td></td>
<td>Very low</td>
</tr>
</tbody>
</table>

Case 4 – Clinical Summary

Male, age 45
- Liver transplantation for autoimmune hepatitis
- Incidental nodule 1.2cm diameter in right lobe of liver
Case 4 – H & E findings

Well-differentiated hepatocellular lesion showing:

- Cytological atypia
  - Increased N/C ratio, nuclear crowding, mild nuclear pleomorphism
- Architectural atypia
  - Thick plates (>3 cells thick), glandular structures
- Probable microvascular invasion
  - Sufficient to warrant a diagnosis of well-differentiated HCC (versus large cirrhotic nodule or dysplastic nodule)

- What additional stains may be helpful in the biopsy diagnosis of early/well-differentiated HCC?
  - Particularly applies to small lesions (1-2cm) where radiological findings are inconclusive

### Assessment of Well-differentiated Hepatocellular Lesions - Reticulin Staining

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

### Benign Versus Malignant Nodules in Cirrhotic Livers

Features favouring a diagnosis of malignancy

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ki-67</td>
<td>Stepwise increase (cirrhotic nodule – dysplastic nodule – early HCC) No defined threshold for diagnosing HCC Rarely exceeds 5% in well-differentiated HCC</td>
</tr>
<tr>
<td>CD34</td>
<td>Diffuse capillarisation of sinusoids (vs focal in cirrhotic nodules) May also occur in high-grade dysplastic nodules (and in FNH &amp; liver cell adenoma)</td>
</tr>
<tr>
<td>AFP</td>
<td>Rarely positive in early HCC</td>
</tr>
</tbody>
</table>
Benign Versus Malignant Nodules in Cirrhotic Livers
Features Favouring a Diagnosis of Malignancy
More Recent Immunohistochemical Markers

Molecular studies have identified many genes up-regulated in early HCC. Some have products that can be demonstrated immunohistochemically.

<table>
<thead>
<tr>
<th>Gene/Protein Upregulated in Early HCC</th>
<th>Immunohistochemistry (criteria for positivity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glypican-3</td>
<td>Heparan sulphate proteoglycan</td>
</tr>
<tr>
<td></td>
<td>Promotes growth of HCC by stimulating Wnt signaling</td>
</tr>
<tr>
<td></td>
<td>Cytoplasmic/membranous (&gt; 5-10% of cells)</td>
</tr>
<tr>
<td>HSP 70</td>
<td>Hsp70 stress protein</td>
</tr>
<tr>
<td></td>
<td>Promotes anti-apoptotic survival factor</td>
</tr>
<tr>
<td></td>
<td>Cytoplasmic/nuclear (&gt; 5-10% of cells)</td>
</tr>
<tr>
<td>Glutamine Synthetase</td>
<td>Glutamine synthetase</td>
</tr>
<tr>
<td></td>
<td>Overexpressed with activation/mutation of beta-catenin</td>
</tr>
<tr>
<td></td>
<td>Involved with hepatocyte regeneration/proliferation</td>
</tr>
<tr>
<td></td>
<td>Cytoplasmic (&gt;50%, unrelated to vessels)</td>
</tr>
</tbody>
</table>

Ki 67
(10-15% pos)

CD 34
AFP
Glypican 3
Glutamine Synthetase
Benign Versus Malignant Nodules in Cirrhotic Livers
Features Favouring a Diagnosis of Malignancy – Recent 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, Tremonti 2012)

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

---

**Case 4 – Diagnosis**

Hepatocellular carcinoma (well-differentiated) arising in a background of cirrhosis (ABH)

**Case 5 – Clinical Summary**

Female, age 26
- Right hemi-hepatectomy specimen.
- Large mass 12 cm diameter with central scar. Enlarged perihilar and coeliac lymph nodes.
- Slide submitted is from periphery of lesion to include surrounding non-neoplastic liver tissue.
Tumour Periphery
(including adjacent non-lesional liver)

Tumour Centre

Hep Par 1
Case 5 – Histological Findings

- Well-differentiated hepatocellular lesion
- Large cells with abundant granular eosinophilic cytoplasm
  - "Oncocytic-like" appearance (due to presence of numerous mitochondria)
- Large nuclei and nucleoli
- Dense stroma arranged as parallel lamellae
  - Most abundant centrally
- Immunostaining positive for Hep Par 1, pCEA (canalicular), mitochondria and CK7

Case 5 – Diagnosis

Fibrolamellar hepatocellular carcinoma
Case 5 – Discussion Points

Fibrolamellar carcinoma versus conventional HCC

1. Clinico-pathological features
2. Tumour biology
3. Problems with histological diagnosis

Fibrolamellar versus Conventional HCC

<table>
<thead>
<tr>
<th>Clinicopathological Features</th>
<th>Fibrolamellar HCC</th>
<th>Conventional HCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>5-35 years (median 20)</td>
<td>Most &gt; 50 years</td>
</tr>
<tr>
<td>Sex</td>
<td>M-F</td>
<td>M-F</td>
</tr>
<tr>
<td>Aetiology</td>
<td>Unknown</td>
<td>HBV, HCV, alcohol, haemochromatosis</td>
</tr>
<tr>
<td>Uninvolved liver</td>
<td>Normal</td>
<td>Usually cirrhotic (Cases occurring in non-cirrhotic liver usually have risk factors for chronic liver disease and pre-cirrhotic fibrosis)</td>
</tr>
</tbody>
</table>

Fibrolamellar versus Conventional HCC

1. Molecular pathology
   - Differences in gene mutations and molecular signalling pathways
     e.g. FLC not associated with p53 or beta-catenin mutations, elevated AFP or elevated survivin expression (Lu 2009, Malouf 2012)

2. Immunohistochemical phenotype
   - FLC more frequently expresses CK 7, EMA
   - FLC less frequently expresses AFP, beta-catenin, CK 19
   - Hep Par 1, pCEA (canalicular) and CD34 (sinusoidal) typically present in both FLC and conventional HCC

Fibrolamellar HCC - Problems with Histological Diagnosis

1. FLC may contain areas resembling conventional HCC
   - Mixed features of FLC and HCC present in up to 25% cases initially diagnosed as FLC (Malouf 2012)
   - Cases of “mixed FLC” more frequently occur in older people, have higher AFP levels and worse prognosis.
   - Different patterns of recurrence/metastases (mixed FLC – intrahepatic, pure FLC – extrahepatic)

2. Conventional HCC may contain areas with a resemblance to FLC (sclerosing or scirrhous HCC)
   - Mixed features of FLC and HCC present in up to 25% cases initially diagnosed as FLC (Malouf 2012)
   - Cases of “mixed FLC” more frequently occur in older people, have higher AFP levels and worse prognosis.
   - Different patterns of recurrence/metastases (mixed FLC – intrahepatic, pure FLC – extrahepatic)

Fibrolamellar - Problems with Histological Diagnosis

2. Conventional HCC may contain areas with a resemblance to FLC (sclerosing or scirrhous HCC)
   - Typical HCCs in cirrhotic liver may contain foci with cytological features and/or stroma resembling FLC
     - Infrequent case reports
     - Functional significance uncertain
HCC with Foci of FLC
Female, age 67. Liver transplant for NASH. 3.5cm nodule in right lobe

HCC (trabecular pattern)
Fibrolamellar carcinoma
"Transitional zone" (HCC/FLC)