Recent Advances and New Entities

Liver Pathology

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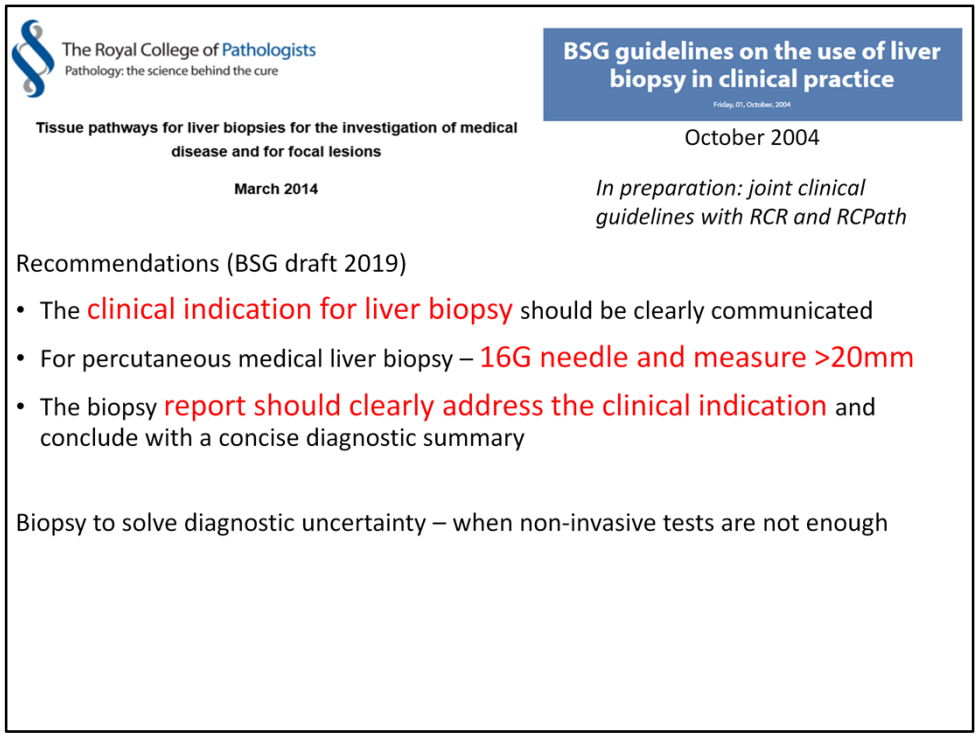
Presentation at the BSG meeting, pathology session, June 18th 2019.

There are few new entities – but considerable changes in the range of biopsies and resections received by histopathologists in the last decade.

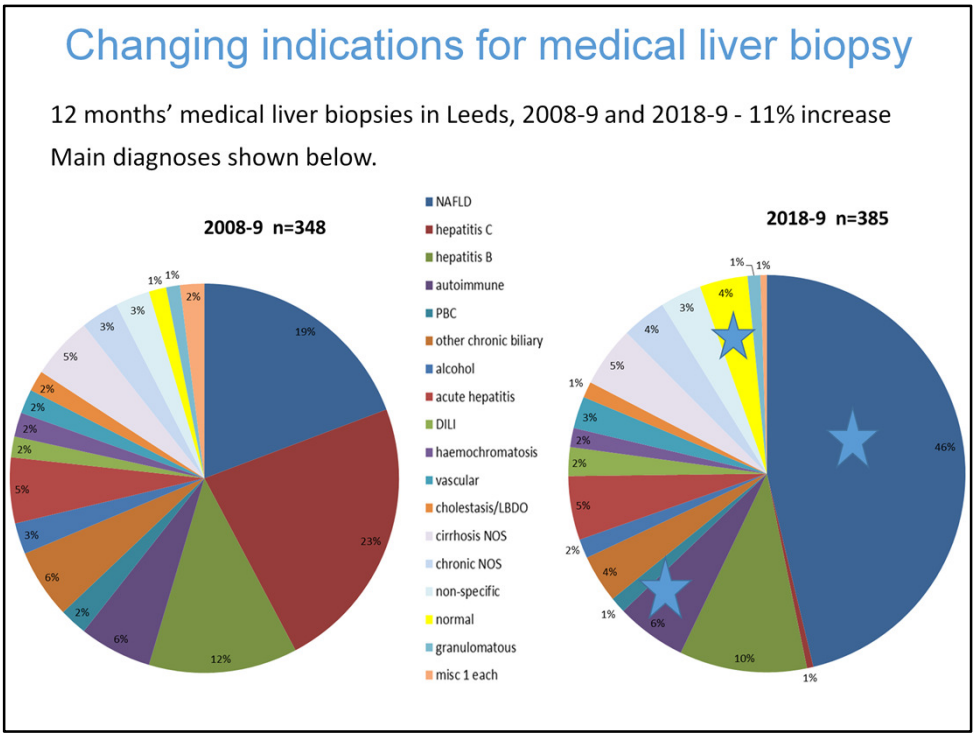
After lots of interesting reading, I chose 6 topics to focus on – 4 medical and 2 tumour.

The next version of the RCPath Tissue Pathways and Liver Dataset are both in preparation, aiming to complete by the autumn this year.



The RCPath Tissue Pathways for liver biopsies is being updated, in tandem with the BSG clinical guidelines on the use of liver biopsy – the BSG guidelines will be a joint document with the Colleges of Radiologist and Pathologists – the previous 2004 document included clinical indications and how biopsies were obtained, but no reference to the pathology.

The draft recommendations of the pathology section of the new guidelines emphasize the importance of there being a clear indication for the biopsy, and that the clinical question is clearly communicated to the pathologist. Biopsies taken for the investigation of medical liver disease should be with a 16 gauge needle, and 20mm long; a second pass should be considered if a shorter core is obtained, especially if the purpose of the biopsy is to determine fibrosis stage or investigate biliary disease. The report should clearly address the clinical indication.



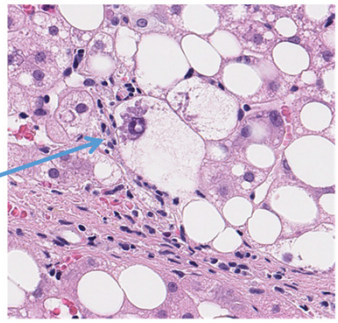
The role of medical liver biopsy has changed in the last 10 years. Biopsies are now very rarely indicated for hepatitis C. Patients are investigated in primary care with the non-invasive liver screen. Patients with a clinical diagnosis of non-alcoholic fatty liver disease have a liver biopsy if they have discrepant non-invasive tests for fibrosis (fibroscan and blood tests e.g. BARD, ELF) or if they have blood tests suggesting an alternative/additional diagnosis e.g. autoantibodies, iron indices. These have more than replaced biopsies for hepatitis C. Other diagnoses have had little change, but there are more biopsies with normal/near normal histology report.

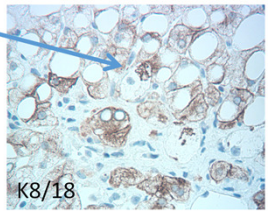
Blue stars – topics included in this presentation. .

Fatty liver disease…….

Histopathology of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis

- Useful information on NASH, includes scoring – for clinical trials and cross-sectional studies.
- NAS score – fat, inflammation, ballooning (NAFLD Activity Score)
- Even one ballooned hepatocyte, in the right context, is enough for steatohepatitis
- Can use IHC if not sure
- Portal inflammation associated with progression





Brown GT and Kleiner DE. Metabolism 2016;65(8):1080-1086

K8/18

A recent useful review of NAFLD. Even one ballooned hepatocyte can swing the diagnosis towards NASH in the correct context. Portal inflammation may be present, is not a required feature for diagnosis, but often associated with progression of disease.

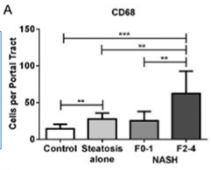
The portal inflammatory infiltrate and ductular reaction in human non-alcoholic fatty liver disease

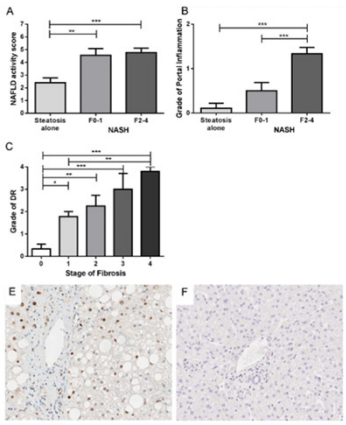
Biopsies from 57 patients with NAFLD

Portal (not lobular) inflammation strongly correlated with fibrosis stage and ductular reaction.

Ductular reaction develops when hepatocyte regeneration is impaired and hepatic progenitor cell proliferation takes over.

Earliest event is portal macrophages in steatosis alone





A: NAFLD acitivity independent of fibrosis
B: portal inflammation increases with fibrosis
C: ductular reaction increases with fibrosis
E,F: p21 senescent hepatocytes

Gadd VL et al. Hepatology 2014:59:1393-1405

More about portal changes in NAFLD. While the ballooning, steatosis and lobular inflammation used to grade activity in NASH do not show a correlation with disease progression, the degree of portal inflammation does increase with fibrosis stage. The degree of marginal ductular reaction around portal areas/septa also increases with the fibrosis stage. This is in keeping with the recruitment of progenitor cells to replenish hepatocytes, as a high proportion of hepatocytes become senescent and unable to enter the cell cycle as the disease progresses. This is consistent with the progressive disease developing in patients who are less able to repair the hepatocyte injury, rather than the severity of the steatohepatitis per se.

The portal inflammation includes many cell types, among which macrophages and T8 lymphocytes are the most numerous. An increase in portal macrophages is the first change observed, and this starts before steatohepatitis develops.

Autoimmune liver disease……

Diagnosis, presentation and initial severity of autoimmune hepatitis in patients attending 28 hospitals in the UK

1267 patients incident since 2007 or prevalent sine 2000 who met 1999 International AIH Group diagnostic criteria (1164) or treated for clinical diagnosis (103).

96% had biopsy, report available in 1163 (92%)

Histological feature	IAIHG histology score (1999)	Of 1163 patients (%)
Interface hepatitis	3	88%
Lympho/plasma cell predominance	1	75%
Rosettes	1	19%
Emperipolesis	0	0.4%
None of above	-5	0.8%
Bile duct changes	-3	2.4%
Steatosis (>mild)	-3	2.3%
Other predominant pathology	-3	1.4%

31% who met 1999 IAIHG criteria did not meet 2008 'simplified criteria

'**typical**' 2 points
- interface hepatitis
- emperipolesis
- rosettes

'**compatible**' – 1 point

Gordon V *et al.* Liver International 2018:38:1686-1695

Many in the audience may be among pathologists whose reports of autoimmune hepatitis (AIH) are included in this study of presentation of autoimmune hepatitis in 28 UK hospitals. The study includes aspects of presentation and diagnosis – this slide refers to biopsy findings, based on a review of the histology reports (no slide review). The authors extracted the elements of the 1999 IAIHG criteria for diagnosis of AIH. Interface hepatitis and lymphocytic/plasma cell predominance are routinely recorded, but rosettes are only recorded by a few pathologists.

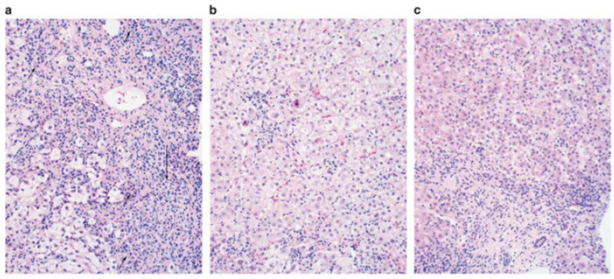
Rosettes and emperipolesis (the appearance of lymphocytes within the cytoplasm of hepatocytes) are not items generally included in the histology report. This affects the AIH score, especially the 'simplified critieria' published in 2008, which was based on the premise that typical AIH is characterised by interface hepatitis, resetting and emperipolesis. If clinicians are using this score, they need to communicate this to their pathologists. However the latter two features (resetting and emperipolesis) are poorly reproducible, see next slide.

Autoimmune hepatitis: review of histologic features included in the simplified criteria proposed by the IAIHG and proposal for new histological criteria

Biopsy is required for AIH diagnosis

IAHG simplified:

For 2 points – interface hepatitis, emperipolesis and rosettes – the latter two poorly reproducible and non-specific



Proposed: interface/lobular activity,
number of plasma cells,
presence/absence of biliary features (copper and CK7 stain).

Increased sensitivity for AIH especially in acute presentation.

Balitzer D et al, Modern Pathology 2017;30;773-783

This problem with the 'simplified criteria' was also addressed in the US – this paper describes an alternative scoring system which emphasises the importance of lobular as well as interface inflammation, and focusses on the number of plasma cells. Also on the need for an absence of biliary features to avoid mistaking PBC for AIH. This approach was better at identifying AIH in cases presenting acutely or with concomitant fatty liver disease. Recognising moderate interface hepatitis or lobular hepatitis, and recognising plasma cells are familiar and more reproducible to pathologist in their routine practice. There are currently guidelines in development for reporting AIH.

Variant syndromes of primary biliary cholangitis

Rather than 'overlap syndrome' IAIHG recommends 'variant syndrome' and categorise according to the dominant entity. (EASL Position statement 2011).

Paris criteria: simultaneous or consecutive –

For AIH: ALT >x5 ULN; IgG >x2 ULN or anti-SMA PLUS biopsy showing moderate of severe periportal/periseptal lymphocytic interface hepatitis

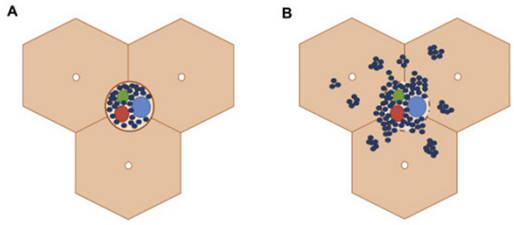
For PBC: AlkP >x2 ULN; GGT >x5; AMA, IgM PLUS biopsy showing florid bile duct lesions

Liver biopsy is mandatory.

Clinicians:

Suspect variant syndrome when patients do not respond to UCDA or when sudden increase in transaminases.

Consider sending biopsy to a reference centre.



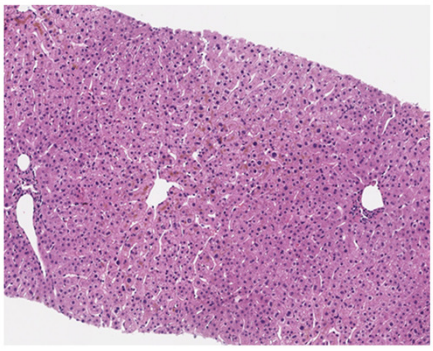
Classic PBC variant PBC

Schulz L et al. Best Practice and Research Clinical Gastroenterology 2018:34-35:55-61

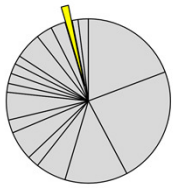
'Overlap' syndromes are a common source of uncertainty. The terminology 'variant syndromes' has been preferred by the IAIHG since this is suggested to better indicate the concept that these are a continuum and not a specific entity. It is usually possible to recognise the predominant disease process.

Biopsy is necessary for diagnosis, indicated when patients do not respond to treatment as expected, and/or have both biochemical and serological features of both diseases. This paper emphasises the contribution of lobular hepatitis as an important distinct component of variant PBC.
It suggests sending biopsies for central review to benefit from (and add to) concentrated pathologists' experience. Since determining dominant process depends on the integration of the blood test results as well as the morphology, all the relevant clinical information needs to be included if biopsies are referred.

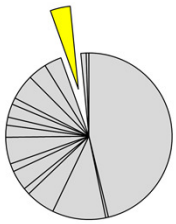
Normal/near-normal liver biopsy ……



2008-9



2018-19



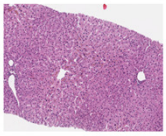
Normal/near normal biopsies – increased from 5 in 2008-9 to 15 in 2018-19

We are reporting more biopsies as normal/near normal – these are usually taken for persistently raised ALT. Such a result is reassuring to the patient and hepatologists, in excluding evidence of any progressive chronic liver disease. What happens to these patients?

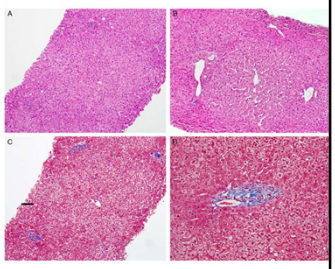
The Almost-Normal Liver Biopsy
Presentation, Clinical Associations, and Outcome

97 almost normal liver biopsies from 2 institutions: (0.6% and 3.7%)

Indications: abnormal LFTs or ?portal hypertension



72% had other medical conditions:
- 18% autoimmune conditions,
- 13% ischaemic events
- 11% metabolic syndrome
- 8% 'drug effects'
- 7% GI tract disease



Median follow up 4.3 years (0-10y) available in 68% patients:
LFTs normalised in half, remained elevated in half

7 patients eventually developed chronic liver disease
AIH or PBC

Liver of a good repairer – resilient to injury

Czeczok TW *et al.* Am J Surg Pathol 2017:41(9);1247-53

A review of 97 biopsies reported as normal/near normal. Patients usually had other medical conditions (the context for testing LFTs?).

For those with follow up, LFTs remained abnormal in half. Seven eventually developed chronic liver disease – 6 had AIH or PBC, one had cirrhosis – it is not clear whether these were from the 68% with median 4.3 year follow up – if so, then >10% of the cohort. So perhaps not so reassuring as first appears.

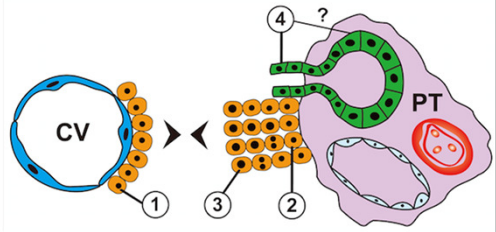
What could this mean – presumably the raised ALT reflects liver injury which is rapidly repaired by a resilient liver, without resulting in inflammatory infiltration or fibrosis.

The many ways to mend your liver: a critical appraisal

Review of recent progress in identifying clonogenic hepatocytes within the liver

4 proposed anatomical locations for hepatocyte stem cells:

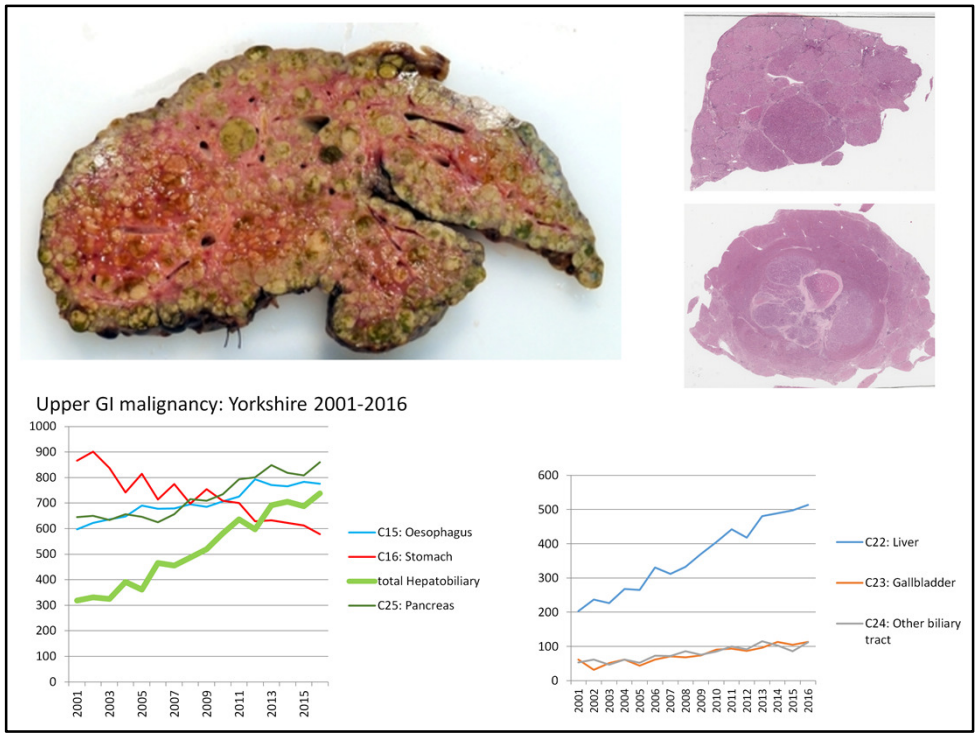
1. Pericentral zone – in chronic liver injury,
2. Periportal zone – compensatory hyperplasia after partial hepatectomy
3. Random distribution, 3-5% hepatocytes with high TERT, acute liver failure
4. Bile duct-derived hepatic progenitor cells – chronically damaged livers with widespread hepatocyte senescence



Allison MR. Int J Exp Pathol 2018;99;106-112

For those interested in how the liver is able to regenerate so well that it grows back in a short time after partial hepatectomy – no other organ can do this.

This short review summarises the four potential stem cell locations in the liver. The ductular reaction which is seen in advanced chronic liver disease of any aetiology represents the activation of bile duct derived progenitor cells, as a response to widespread hepatocyte senescence which is a common denominator of late stage chronic liver disease and cirrhosis.



Patients with cirrhosis are offered surveillance with 6-monthly ultrasound. Cirrhotic livers removed at transplant often show striking variation in the macroscopic appearance of the cirrhotic nodules. Any nodules which are larger or distinctly different from the background need to be sampled for histology; HCC cannot always be recognised macroscopically when small/early.

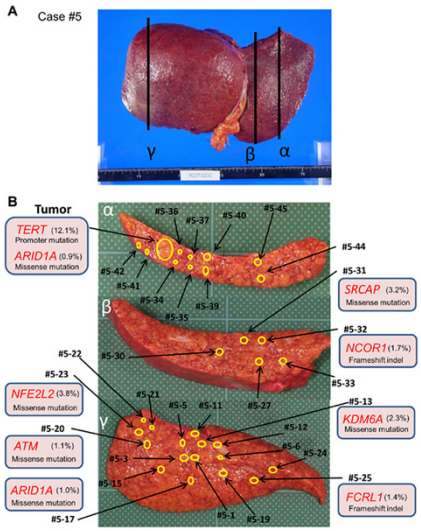
The data from the Yorkshire Cancer Registry shows that primary hepatobiliary cancer has more than doubled in the last 16 years, overtaking gastric cancer, and fast approaching oesophageal and pancreatic cancer in incidence. The increase in hepatobiliary tract malignancy is largely due to HCC.

Examining the explant livers is fascinating – they may contain multiple HCCs and/or dysplastic nodules, which look different from each other – a chance to observe neoplastic transformation in a shared genetic background and aetiology, resulting in the evolution of separate diverse tumours.

Comprehensive analysis of genetic aberrations linked to tumorigenesis in regenerative nodules of liver cirrhosis

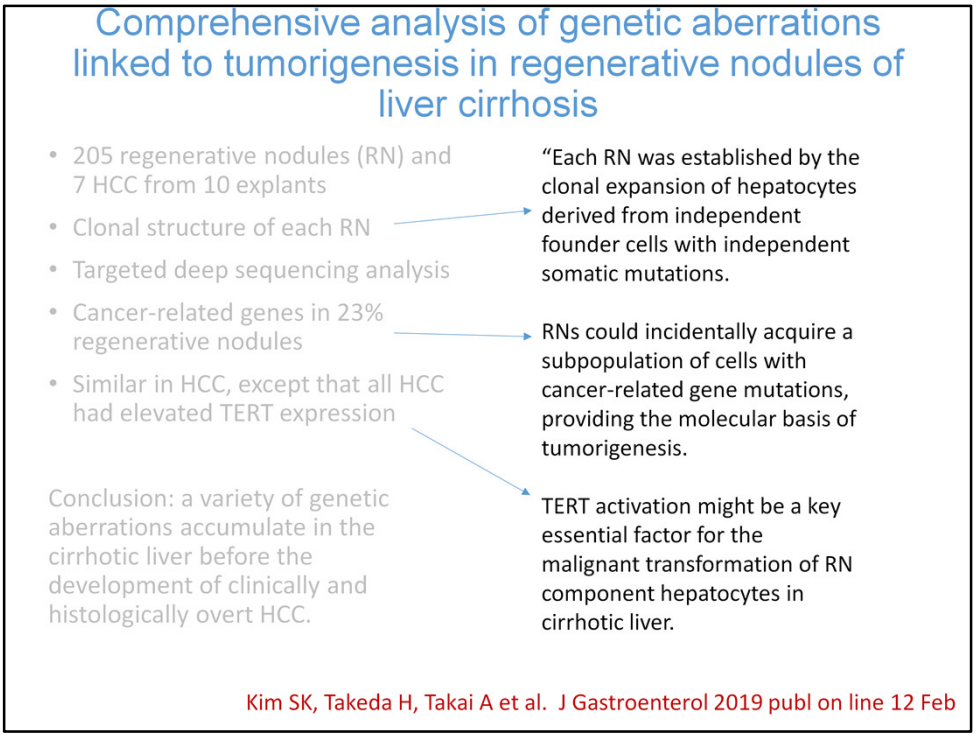
- 205 regenerative nodules (RN) and 7 HCC from 10 explants
- Clonal structure of each RN
- Targeted deep sequencing analysis
- Cancer-related genes in 23% regenerative nodules
- Similar in HCC, except that elevated TERT expression only in HCC (5/7)

Conclusion: a variety of genetic aberrations accumulate in the cirrhotic liver before the development of clinically and histologically overt HCC.

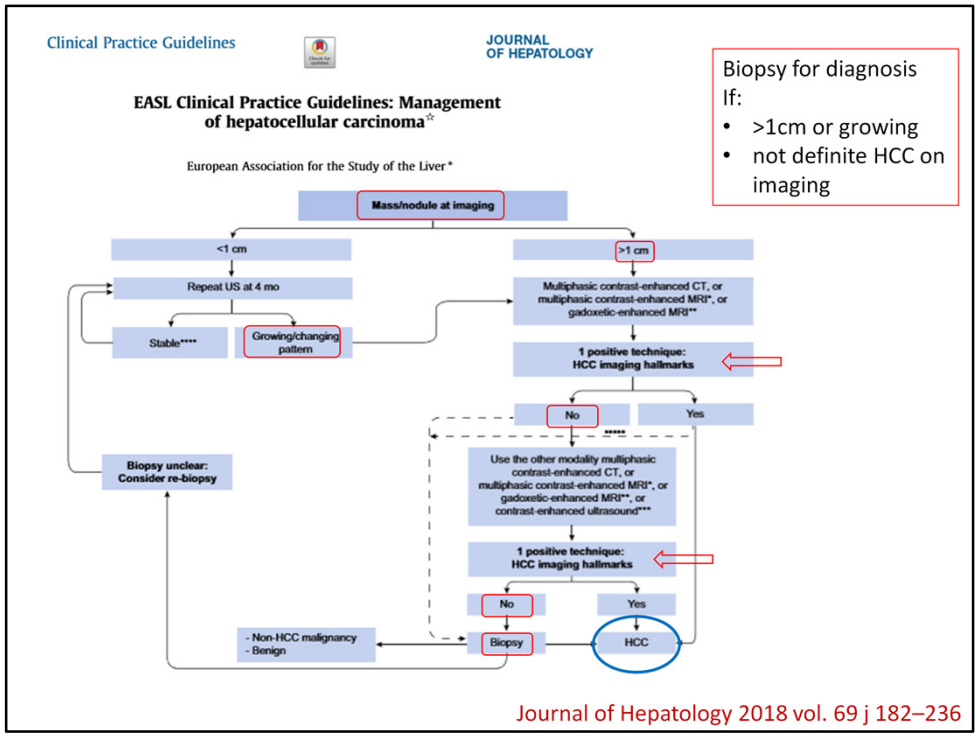


Kim SK, Takeda H, Takai A et al. J Gastroenterol 2019 publ on line 12 Feb

This meticulous Japanese study analysed the genetics of regenerative nodules in 10 explant livers (8 for Hepatitis C, 1 hepatitis B, 1 alcohol related liver disease). Dysplastic nodules were excluded; 7 of the patients had an HCC. They demonstrated by targeted deep sequencing that the regenerative nodules were clonal proliferations; nearly a quarter of them harboured at least one genetic abnormality known to be common in HCC. These were not more common in patients who also had HCC, and were not clustered in the vicinity of the HCC but distributed randomly in the liver.



The single alteration that was detectable only in the HCC and not regenerative nodules was telomerase reverse transcriptase promotor mutation – this would act to confer long term survival on nodules which otherwise have limited duration.



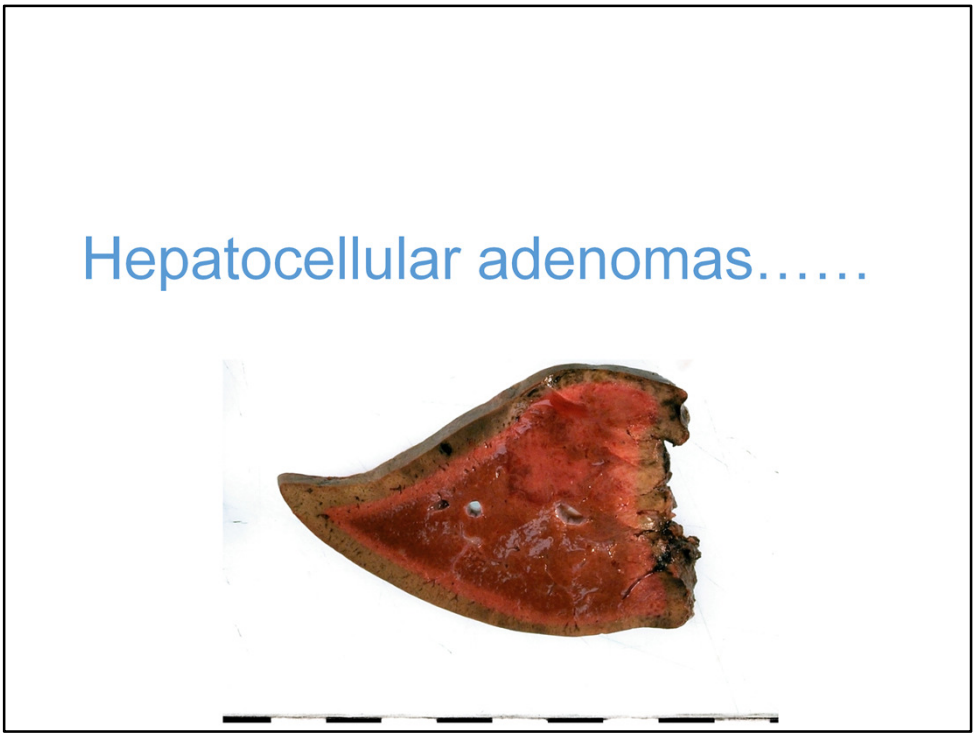
Patients with cirrhosis are offered surveillance aimed at detecting HCC while early and treatable. These are the EASL guidelines from 2018 – pathway for diagnosis of HCC in surveillance-detected lesions.

Diagnosis is by MRI or CT imaging of nodules >1cm or changing nodules.

For lesions which do not show hallmarks of HCC on CT/MRI, an alternative technique is used. If still indeterminate, then the next step is to biopsy the lesion.

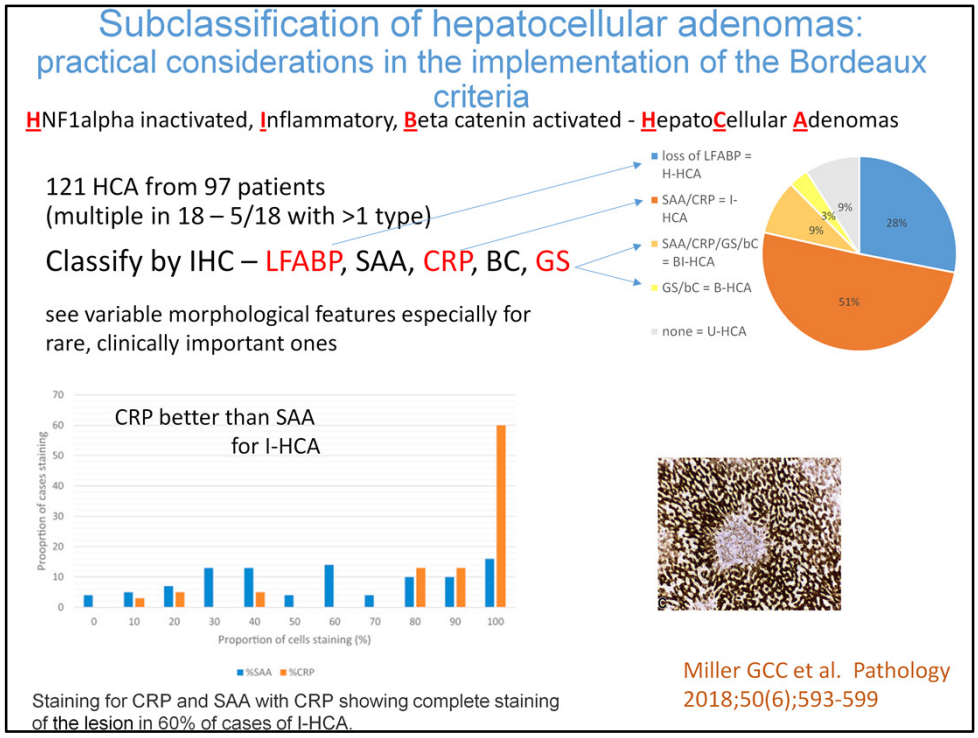
The previous advice against biopsy for HCC diagnosis is revised in this situation. Lesions for biopsy on imaging either have the differential either of 'dysplastic nodule' or are definitely malignant, but not characteristic for HCC (i.e. suggests cholangiocarcinoma or metastasis).

This is resulting in more needle biopsies for ?HCC, which can be very challenging. The risk of causing dissemination of the lesion by biopsy is now considered to have been overestimated in the past.



Hepatocellular Adenomas (HCA)– the classification based on genetic alterations is continuing to evolve.

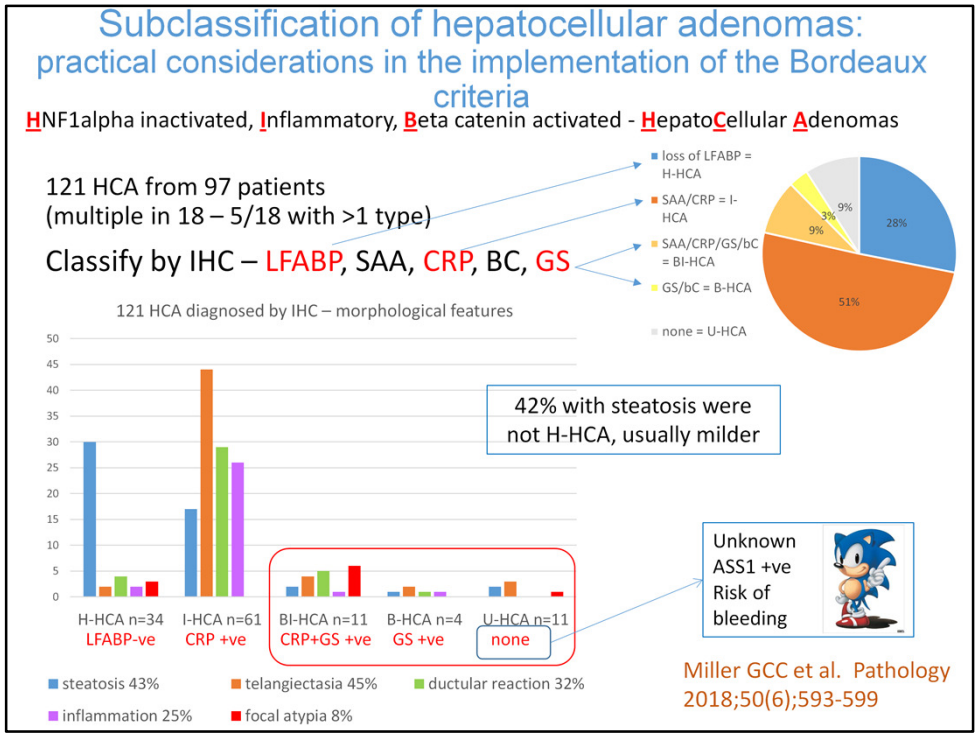
Since genetic analysis is not available routinely in the UK, morphology and immunohistochemistry are used as surrogates with which we can effectively diagnose most HCA.



Most publications have been from the French group that has pioneered the developments in our understanding of HCA.

This publication from Australia describes a pragmatic approach to diagnosis when molecular tests are not available. They conclude that 3 immunostains enable diagnosis; CRP is superior to serum amyloid A.

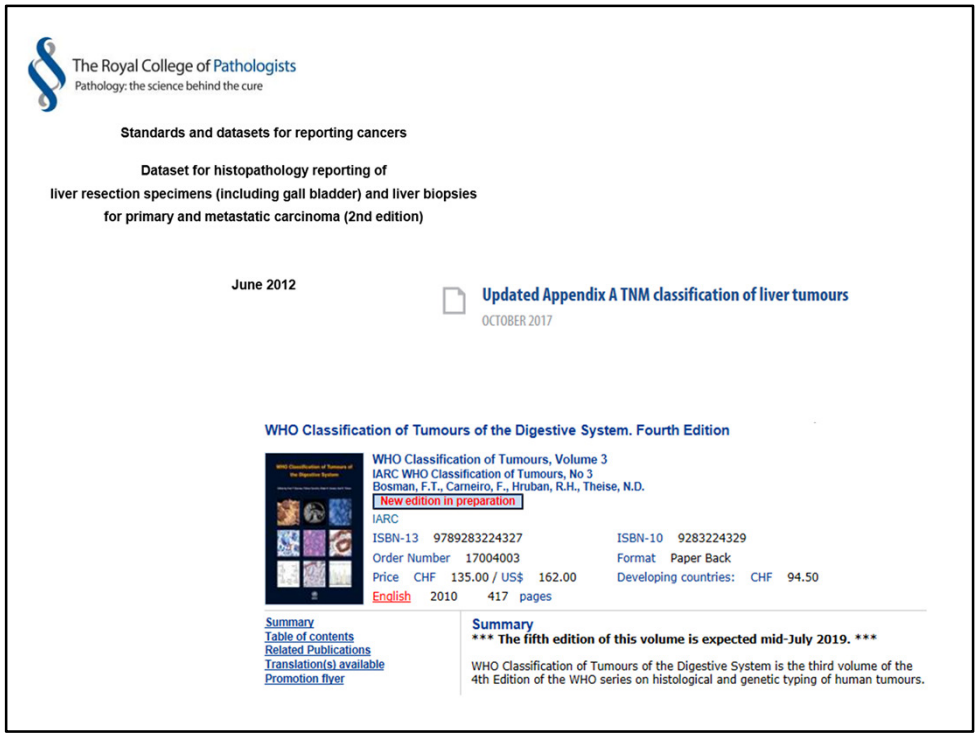
In cases with multiple HCA, these were of more than one type in 28% patients.



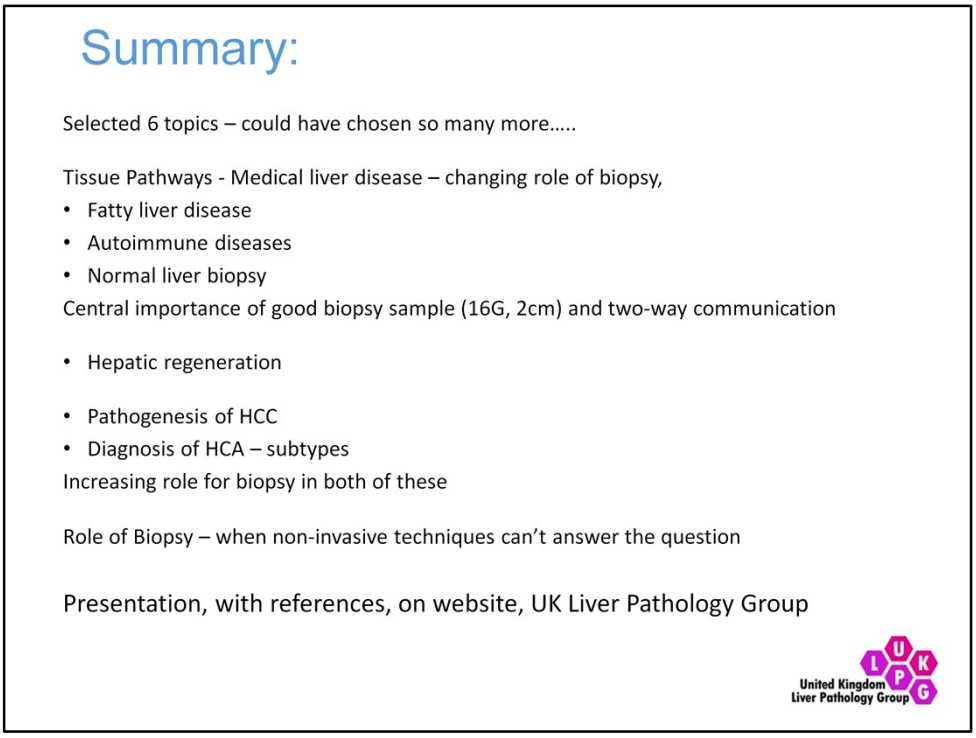
Immunohistochemistry rather than morphology is important to enable recognition of the clinically important types of HCA. These are the beta-catenin activated adenomas, (these may also have activation of the JAK/STAT inflammatory pathway (combined BI-HCA)) - the lesions at higher risk of malignant transformation. Also the unclassified adenomas – the latter are now known to include the newly recognised adenomas with a mutation of the sonic hedgehog pathway. These have a high risk of haemorrhage. In future it is suggested that this may be identified by argininosuccinate synthase 1 – not currently available.

These rare and clinically important HCAs may not have the distinctive morphology of atypia/rosettes, but may show steatosis or features of inflammatory adenoma (telangiectasia, ductular reaction, inflammatory infiltrate) and so risk being missed unless the immunohistochemistry panel is used

Currently biopsy is rarely used in diagnosis; the lesions are resected where possible. In future, biopsy diagnosis may become more common, with a view to identifying lesions which can be managed conservatively.



The next edition of the RCPath Liver Dataset is in preparation – this will be finalised after the WHO Blue Book is published, anticipated in July 2019.



This presentation covered 6 topics representing evolving areas in liver pathology.

Overall, both for medical and oncological indications, the role of biopsy is becoming more clearly defined, providing essential diagnostic information where there is no non-invasive alternative.