SELECTED SUMMARIES

LIVER PATHOLOGY

The aim of these Selected Summaries columns is to concentrate on matters relevant to diagnostic histopathology. With this in mind, the articles I have chosen to cover are based largely on light microscopic observations and deal with aspects which cause diagnostic difficulty to the practising ‘hepatopathologist’. Although there have been many other important advances in hepatology during the past year (notably the development of a serological marker for hepatitis C infection), most of these do not, as yet, have any direct practical application to the interpretation of liver biopsies.

The first three articles deal with primary hepatic neoplasms.

Biopsy diagnosis of well-differentiated hepatocellular carcinoma based on new morphologic criteria. F. Kondo, K. Wada, Y. Nagato, et al. Hepatology 1989; 9: 751–755.

Advances in imaging have enabled the detection of small nodular lesions in the liver, including minute hepatocellular carcinomas (HCCs). These neoplasms are often very well differentiated and, in the cirrhotic liver, are difficult to distinguish histologically from large regeneration nodules.

The histological findings are described in 123 patients with chronic liver diseases and space-occupying lesions in the liver, subjected to ultrasonically guided needle biopsy. Fourteen cases were classified as well-differentiated HCC. Ten of the 14 had lesions less than 2 cm in diameter. These lacked the features of classical HCCs, retaining a normal trabecular arrangement and showing minimal nuclear atypia. The diagnosis was based on three main findings: (i) nuclear crowding in cell cords, (ii) increased cytoplasmic basophilia, and (iii) microacinar formation. The biopsy diagnosis of malignancy was supported by the finding of invasive tumour in subsequent resection or autopsy specimens and/or clinical evidence of tumour progression. Immunostaining for alpha-fetoprotein was unhelpful. The authors felt that the histological features were distinctive from classical HCCs (94 cases), on the one hand, and from large regeneration nodules (9 cases), on the other. However, five cases with uncertain malignant potential were classified as ‘borderline’.

The natural history of small hepatocellular carcinoma remains uncertain, but an accurate histological diagnosis is clearly of fundamental importance in determining prognosis and treatment. The criteria cited in this paper concern the identification of well-differentiated HCC in the cirrhotic liver, but these would be worth applying to the problem of distinguishing HCC from other benign hepatocellular lesions such as liver-cell adenoma.

Well-differentiated cholangiocarcinoma: diagnostic significance of morphologic and immunohistochemical parameters. T. Nakajima and Y. Kondo. Am J Surg Pathol 1989; 13: 569–573.

Intrahepatic cholangiocarcinomas (CCs) are often well-differentiated and are difficult to distinguish from benign proliferations of biliary epithelium, particularly in cases associated with biliary obstruction.

Detailed histological analysis, mucin histochemistry, and immunohistochemical staining were carried out in 62 cases of well-differentiated cholangiocarcinoma (34 peripheral, 28 hilar). Histological changes were evaluated in areas showing overt invasive growth or in metastases and compared with non-cancerous biliary epithelium distant from the tumour. Numerous features were assessed and subjected to multivariate statistical analysis. Not surprisingly, no single criterion could be used to distinguish malignant from benign biliary epithelium. Three major factors were found to be useful in the diagnosis of cholangiocarcinoma: (i) nuclear size variation (nuclear size more than twice normal), (ii) formation of a second gland (presence of intracytoplasmic lumina in individual biliary epithelial cells or intraluminal cribriform structures), and (iii) positive immunostaining for CEA.
these features was occasionally present in non-cancerous biliary epithelium, the findings of two or more was confined to CC and present in 54 of the 62 cases (87 per cent). Other features with a high diagnostic specificity were irregular nuclear configuration, mitoses, and prominent nucleoli. It is suggested that these might be applied in cases where only one of the three major criteria is present.

Malignant epithelioid haemangioendothelioma of the liver: a clinicopathological and histochemical study of 12 cases. O. Dietze, S. E. Davies, R. Williams and B. Portmann. Histopathology 1989; 15: 225–237.

Following the first description of epithelioid haemangioendothelioma (EHE) as a primary hepatic neoplasm in 1984, this unusual tumour has been the subject of considerable interest. Several reports have appeared in the literature and one has the impression that this may represent a genuine increase in incidence. Behaviour is unpredictable but EHE appears to occupy an intermediate position between the fully benign haemangioma and highly malignant angiosarcoma of the liver.

The clinical and pathological findings are described in 12 cases of EHE. Six patients were male, six female, with an age range of 12–54 years (median 30 years). Seven had evidence of extrahepatic spread at, or shortly after, referral and six have died of liver failure due to massive tumour infiltration.

Histological features were evaluated in six needle biopsies, six wedge biopsies, two hepatectomy specimens obtained at liver transplantation, and three autopsy specimens. The authors emphasize the presence of a wide range of histological appearances with three main patterns being identified. Pattern (1), corresponding to the advancing edge of the tumour, showed neoplastic cells scattered in small groups between fairly normal liver cell plates. Pattern (2) showed a complex mixture of pleomorphic tumour cells and atrophied liver cell plates in a scanty fibrous stroma. Pattern (3) showed a predominance of fibromyxoid stroma with only sparse tumour cells present. These three patterns were intermingled in most cases, but showed a concentric arrangement in others. Two types of vascular invasion were noted—tuft-like intra-vascular proliferations of epithelioid cells and fibrothrombotic venous occlusions.

Detailed immunohistochemical findings are not presented, but staining for factor VIII related antigen and CAM5.2 were said to be useful in distinguishing tumour cells from hepatocytes respectively.

It is important to recognize the diverse histological appearances of EHE, particularly in evaluating needle biopsies with the attendant problems of sampling. The actively growing phase, corresponding to pattern (1) above, may mimic other malignant processes including metastatic carcinoma; whereas the sclerotic phase, corresponding to pattern (3) above, may be mistaken for benign vaso-obliterrative or other scarring processes. Although histology is the prerequisite for a correct diagnosis, it appears to have little value in predicting outcome.

It is well known that a pattern of liver damage closely resembling that seen in alcoholic liver disease sometimes occurs in people who deny alcohol consumption. This condition, which has been referred to by a variety of terms, including fatty liver hepatitis and non-alcoholic steatohepatitis, is most commonly associated with obesity and diabetes mellitus. Jejuno-ileal bypass operations and various drugs other than alcohol are implicated in a minority of cases. The following two papers give a further insight into the incidence, histological features, and natural history of ‘non-alcoholic, alcoholic liver disease’.

Alcohol-like liver disease in non-alcoholics. A clinical and histologic comparison with alcohol induced liver injury. A. M. Diehl, Z. Goodman, and K. G. Ishak. Gastroenterology 1988; 95: 1056–1062.

In a review of 129 biopsies initially diagnosed histologically as alcoholic hepatitis, 39 were reclassified as non-alcoholic on the basis of a repeated, unequivocal denial of alcohol consumption. The clinical, biochemical, and histological findings in these cases were reviewed and compared with 68 cases known to be alcoholic.

Significant clinical and biochemical differences were observed between the two groups. Alcoholic patients were more frequently symptomatic (88 vs. 23 per cent), had significantly higher levels of serum transaminases and bilirubin, and significantly lower levels of serum albumin. Although clinical manifestations were generally mild in the non-alcoholic patients, four had evidence of portal hypertension and one died from liver-related causes.

Overall, no qualitative histological differences were observed between the two groups. A full spectrum of alcoholic lesions was seen in the non-alcoholic group, including cirrhosis in ten cases. A number of quantitative differences were noted.
Fatty change was more severe in the non-alcoholic cases, whereas lobular inflammation, Mallory body formation, and fibrosis were all more pronounced in the alcoholics. There was no significant difference in the incidence of cirrhosis.

Non-alcoholic steatohepatitis: a study of 49 patients.
R. G. Lee. Hum Pathol 1989; 20: 594–598.

In a review of 543 liver biopsies initially diagnosed histologically as alcoholic hepatitis, 49 cases were reclassified as non-alcoholic steatohepatitis (NASH). Associated conditions were obesity (> 130 per cent of ideal body weight), present in 34 patients, and diabetes mellitus, present in 25 patients. One patient developed clinical evidence of liver failure and died from a variceal bleed.

The histological appearances in the initial biopsies from these cases were, by definition, indistinguishable from alcoholic hepatitis. The entire morphological spectrum was represented, including cirrhosis in eight patients. Using a semi-quantitative scoring system, histological abnormalities were generally graded as mild-to-moderate in severity. A few minor differences from classical alcoholic hepatitis were observed. Although neutrophils were present in all cases, mononuclear cells were frequently admixed and predominated in 21. Mallory bodies were identified in less than 50 per cent and were generally sparse. Glycogenated nuclei were seen in 17 cases, 14 of whom had a history of diabetes mellitus.

Follow-up biopsies were available in 13 cases, over a period of 1.2–6.9 years. Eight of these showed no obvious progression. Five showed progressive fibrosis, with development of cirrhosis in two.

Both of these studies show a considerable overlap between the histological features of alcoholic hepatitis and those of non-alcoholic steatohepatitis. This is hardly surprising given the way in which the cases were selected. As regards liver biopsy interpretation, it is clearly not possible for the histopathologist to identify with certainty cases in which a cause other than alcohol is responsible for fatty liver hepatitis. There remains the suspicion that patients labelled as having NASH may be covert alcoholics, but differences in clinical presentation and natural history in these studies suggest that NASH may be a separate clinicopathological entity. Although NASH is generally regarded as a relatively benign condition, 20 of 88 patients studied in these two papers developed cirrhosis, and two died from liver-related complications.

The final three papers I have selected deal with aspects of liver transplantation. Although the interpretation of post-transplant liver biopsies is still largely the province of a small number of pathologists, there is little doubt that exposure to these specimens will widen as the number of transplant operations continues to increase.

The first two papers deal with the histological diagnosis of acute rejection in the liver allograft. Acute rejection is a common complication of liver transplantation, and the histological features have been well described in several recent papers. Typically there is a triad of portal inflammation, bile duct damage, and venous endothelial inflammation which respond to treatment with high-dose steroids. The histological diagnosis is not always straightforward, particularly when the changes present are mild or when rejection co-exists with other graft complications. These two papers attempt to define the most reliable criteria for diagnosing acute rejection using statistical analysis.

An analysis of the determinants of hepatic allograft rejection using stepwise logistic regression. H. Sankary, P. Foster, M. Hart, M. Ashmann, D. Schwartz and J. W. Williams. Transplantation 1989; 47: 74–77.

Five hundred and sixty-six allograft biopsies were examined in 56 patients following liver transplantation. Thirty-nine biopsies were classified as showing acute rejection on the basis of a portal tract mononuclear cell infiltrate associated with spillover into the parenchyma. These biopsies were obtained from patients with graft dysfunction in whom technical problems had been excluded by other investigations. The remaining 527 biopsies, from patients without rejection, included 127 with other forms of graft dysfunction. Thirty-five histological variables were examined and graded semi-quantitatively on a scale of 0–3. The results were analysed by stepwise logistic regression in an attempt to construct a mathematical model for predicting rejection.

The final model selected six variables which could reliably predict rejection. These were (i) portal tract spillover, (ii) portal tract eosinophilia, (iii) portal vein endothelialitis, (iv) portal tract neutrophilia, (v) central vein endothelialitis, and (vi) cholestasis.

The study is open to some criticisms. The nature of graft dysfunction in the 127 biopsies without rejection is not stated. No attempt is made to grade
the overall severity of rejection present. I was also surprised to find that bile duct inflammation was not identified as a reliable predictor, as most studies indicate this to be a diagnostic feature of acute rejection. Nevertheless, the findings are interesting, particularly the demonstration of neutrophils and eosinophils as a significant component of the portal infiltrate in acute rejection. There is increasing evidence to suggest that these cells play an important role in augmenting immune-mediated damage to target structures in liver allografts, and are not merely present as innocent bystanders.

**Bile duct injury as a part of diagnostic criteria for liver allograft rejection.** J. Kemnitz, B. Ringe, T. Cohnert, G. Gubernatis, H. Choritz and A. Georgii. *Hum Pathol* 1989; 20: 132–143.

This study specifically examines the relationship between bile duct damage and other histological features of acute rejection. Three hundred and twenty-nine biopsies with a histological diagnosis of rejection were examined. In all cases there were accompanying clinical signs of rejection. The overall severity of rejection, the degree of cholestasis, and the severity of changes in interlobular bile ducts were graded semi-quantitatively on a scale of 0–3 and the results analysed statistically.

Not surprisingly, the severity of bile duct damage correlated with the overall severity of rejection (bile duct damage was used as one of the features in grading rejection). More interestingly, the authors conclude that bile duct injury is a more sensitive indicator of acute rejection than venous endothelial damage. Bile duct injury, albeit mild, was a consistent finding in cases with borderline histological rejection (classified as grade 0–1) in which there were clinical signs of graft dysfunction, but where venous endothelialitis was absent. Another interesting observation was the finding that the severity of cholestasis correlated with the severity of acute rejection—although the two lesions frequently coexist, the precise relationship between cholestasis and rejection has never been fully established. The authors suggest that there may be a causal relationship between bile duct damage and cholestasis in acute rejection. This association is more readily apparent in cases of chronic rejection where there is a progressive destruction of small bile ducts (the so-called 'vanishing bile duct syndrome').

The final paper deals with a question that has caused considerable controversy amongst clinicians and pathologists dealing with liver transplant patients. Does primary biliary cirrhosis (PBC) recur after transplantation? From a pathological point of view, the main problem has been in distinguishing recurrent PBC from chronic rejection—both conditions are characterized by portal inflammation and the progressive destruction of small intrahepatic bile ducts. Most cases of chronic rejection result in graft failure within the first year after transplantation. Given the slow evolution of PBC in the non-transplanted liver, the question of recurrent disease clearly needs to be addressed over a much longer follow-up period.

**Evidence for disease recurrence after liver transplantation for primary biliary cirrhosis. Clinical and histologic follow-up studies.** R. J. Polson, B. Portmann, J. Neuberger, R. Y. Calne and R. Williams. *Gastroenterology* 1989; 97: 715–725.

This paper is a follow-up to a previous report from the same group in which evidence was presented for the recurrence of PBC in three patients after transplantation. Twenty-three patients with PBC who survived more than 1 year after transplantation were studied and compared with 102 non-PBC patients who survived for a similar period of time. Ten patients in the PBC group and 50 in the non-PBC group had biopsies taken after 1 year (22 and 75 biopsies, respectively; range 1–11 years). Nine of the ten PBC patients had biopsies showing features compatible with recurrent disease. These included the presence of bile duct distortion (eight cases), breaks in the basement membrane of bile ducts (five cases), and paucity of bile ducts (two cases). Additional findings were portal lymphocytic aggregates (seven cases), granulomas (three cases), and copper-associated protein (CAP) (four cases). Biopsies were classified as stage I in seven cases and stage II or III in the other two. Four patients also had clinical and biochemical features suggestive of PBC recurrence. By comparison, only 12 of the 50 non-PBC patients had histological evidence of bile duct damage. None showed breaks in the basement membrane of bile ducts, granuloma formation, or CAP deposition, and in each case there were clinical, biochemical, and histological features supporting an alternative diagnosis.

This study provides further evidence that recurrent PBC can be observed after transplantation, provided that patients are followed up over a long
enough period. Previous studies which claim that PBC does not recur have failed to do this and I think it is likely that we will be seeing more cases of recurrent PBC in the future.

S. G. HUBSCHER
Department of Pathology
University of Birmingham
Birmingham B15 2TJ