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The pathology of the atrophy/hypertrophy complex (AHC) of the liver. A light microscopic and immunohistochemical study

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Summary. The term, atrophy/hypertrophy complex (AHC) of the liver, denotes a distinct combination of hepatic atrophy and hypertrophy occurring in situations of significant impairment of bile flow and/or portal or hepatic venous blood flow. In the lobes or segments concerned atrophy ensues, whereas areas not or less involved develop compensatory hypertrophy, resulting in a characteristic gross deformity of the organ and, in some instances, in rotation of the liver around a virtual hilar axis. As recognition and early detection of AHC have a strong implication on the treatment of several hepatobiliary diseases, adequate combined clinical, radiological and histopathological strategies have to be used in order to arrive at a correct diagnosis. The present investigation was designed to analyze the morphology of AHC in detail and to define lesion patterns having the highest predictive value. For atrophy, the following features were highly characteristic: 1) Advanced septal fibrosis with or without nodular change of parenchyma; 2) Biliary piecemeal necrosis with formation of vascular structures; 3) Ductular proliferations, frequently extending into septa and involving the parenchyma; 4) Capillarization of sinusoids with type IV collagen deposition in Disse's space; 5) Factor VIII-associated antigen expression by sinusoidal endothelia; 6) a seemingly paradoxical increase of proliferative activity of hepatocytes as based on PCNA staining. The severity of lesions in atrophy was related to the type of underlying disease, in that the changes were clearly more expressed in situations of longstanding obstruction due to benign disease. Using a set of well-defined morphological parameters, atrophy can be reproducibly distinguished from hypertrophy in biopsy material from AHC.

Key words: Atrophy, Hypertrophy, Liver

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Introduction

Atrophy of the liver has long been known to occur due to old age or to external pressure (e.g., the rib cage), and may ensue after infectious or toxic liver damage (Arakawa, 1965; Von Frerichs, 1969; Lageron and Caroli, 1970). In contrast, the particular combination of atrophy and hypertrophy of the liver, termed atrophy/hypertrophy complex (AHC), has been recognized in recent years only (Benz et al., 1952; Barbier and Van der Stricht, 1969; Kobylinski, 1971; Tsuzuki et al., 1973; Ham, 1974; Weichert et al., 1979; Czerniak et al., 1986; Hadjis et al., 1986a,b, 1987, 1989; Hadjis and Blumgart, 1987, 1988; Matthews et al., 1991). In ACH, hepatic areas with a significant impairment of bile flow and/or portal or hepatic venous flow may develop important atrophy of the region concerned, whereas areas not or less involved may then show considerable compensatory hypertrophy resulting in a characteristic gross deformity of the organ and, in some instances, in a rotation of the liver around a virtual hilar axis. The pathogenesis of AHC has not been elucidated so far, but its recognition and early detection have a strong implication on the treatment of several hepatobiliary diseases (Hadjis et al., 1986a,b, 1989; Hadjis and Blumgart, 1987, 1988; Matthews et al., 1991; Schweizer et al., 1994). Using an interdisciplinary approach it has recently been demonstrated than an efficient strategy can be employed to identify patients with AHC within a clinical setting and to arrive at an appropriate treatment plan (Schweizer et al., submitted). In addition, the latter investigation has shown that preoperative histological stages of AHC can be defined, which may have in impact on the evaluation of prognosis.

The aim of the present retrospective study was to analyze the morphology of AHC in more detail. In particular, we attempted to define those lesions (or patterns thereof) occurring in biopsies having the highest predictive value for either atrophy or hypertrophy.

Materials and methods

Patients

The present investigation is based on liver tissues from 14 patients, in whom AHC had been diagnosed clinically and radiologically between 1987 and 1989. The clinical details of these patients are reported elsewhere (Schweizer et al., submitted), but the most relevant data are compiled in Table 1. In an additional patient, AHC was found at autopsy. The set of hepatobiliary disorders resulting in AHC in the total of 15 patients comprised: benign stricture of bile ducts (6/15); cholangitis (4/15); echinococcus alveolaris (1/15); and neoplastic stenosis of bile ducts (4/15; 1 Klatskin tumor, 1 carcinoma of the cystic duct, 2 carcinomas of the pancreatic head).

Material for histology

For histological analysis, a total of 20 tissue samples were available (16 from macroscopically atrophic, and 4 from macroscopically hypertrophic areas, according to clinical and radiological evaluation). Based on this material, a direct comparison between clinically atrophic and hypertrophic areas was possible in three situations only. In 11 cases only tissue from the atrophic or hypertrophic parts was available for study. Overall, the tissue samples contained 981 assessable portal tracts.

For light microscopy, tissue was fixed in buffered neutral formaldehyde solution (4%) throughout. Dehydrated samples were embedded in paraffin, and tissue sections were stained with haematoxylin and eosin (H&E). The reason why we based our investigation on H&E-stained sections only was that we attempted to test as to whether reproducible parameters for detecting AHC could be worked out with preparations that are available in almost any situation.

Table 1. AHC study: patients

PATIENT NUMBER	AGE (yrs)	SEX	UNDERLYING DISEASE LEADING TO AHC
1	49	f	Benign bile duct stricture
2	45	m	Benign bile duct stricture
3*	25	m	Recurring cholangitis
4	43	f	Alveolar echinococcus
5	68	f	Recurring cholangitis
6	75	f	Benign bile duct stricture
7	75	m	Klatskin tumor
8*	72	m	Carcinoma of cystic duct
9	41	f	Recurring cholangitis
10	68	f	Benign bile duct stricture
11	53	f	Benign bile duct stricture
12	47	f	Recurring cholangitis
13	47	f	Benign bile duct stricture
14	49	f	Carcinoma of the pancreatic head
15	76	m	Carcinoma of the pancreatic head

*: 2 biopsies.

Immunohistochemistry

Immunohistochemical studies were performed in order to assess as to whether this approach would provide relevant information for detecting AHC in addition to conventional light microscopy. Antibodies used in the present study are listed in Table 2. Immunohistochemistry was performed using the avidin-biotin-peroxidase (ABC) technique (Hsu et al., 1981), with some modifications. Paraffin sections were mounted using polyvinyl acetate glue (Järvinen and Rinne, 1983), deparaffinized, hydrated, treated with protease, and dipped in skimmed milk (5%) for blocking non-specific binding (Johnson et al., 1984). Sections were then incubated overnight with primary antibodies, followed by incubation with biotinylated antisera and ABC reagents (one hour each; Dakopatts, 1:200). Peroxidase reactivity was visualized with diaminobenzidine as chromogen (Ruchti et al., 1984). After counterstaining with haematoxylin, sections were mounted with aqueous polyvinylalcohol medium. Liver tissue samples from a parallel study of PBC and samples from liver harbouring hepatic tumors were used as non-AHC controls.

Morphological test parameters analyzed in the present study

Atrophy and hypertrophy of liver tissue may show several histological features, but their diagnostic strength has not yet been fully established (Benz et al., 1952; Arakawa, 1965; Lageron and Caroli, 1970; Weinbren et al., 1985; Hadjis and Blumgart, 1987). Therefore, the following set of histological parameters was subjected to systematic analysis:

1. Fibrosis (including portal tract and septal fibrosis, and periductal fibrosis).

Table 2. Antibodies used in the present study.

ANTIBODY	DILUTION	SOURCE	DETECTION OF:
Cytokeratin 19	1:100	Sigma	Bile duct and ductular epithelia
CAM 5.2	Undilut.*	Becton-Dickinson	Hepatocytes, bile duct epithelia
Vimentin	1:10**	Dakopatts	Mesenchymal cells
SMA	1:300	Sigma	Smooth muscle cells, MFBs
COL IV	1:100	Dakopatts	Basement membranes
FVIII-Ag	1:300	Internal	Vascular endothelial cells
PCNA	1:10	DAKO, PC10	Nuclei of proliferating cells

*: protease digestion with 0.02% trypsin (Difco, Trypsin 1:250) at 37 °C for 30 min; **: no protease digestion; CAM 5.2: antibodies directed against cytokeratins 8 and 18; SMA: antibody directed against smooth muscle actin; FVIII-Ag: antibody directed against factor VIII-related antigen; PCNA: antibody directed against proliferating cell nuclear antigen (cyclin).

2. Biliary piecemeal necrosis.
3. Alterations of interlobular and septal bile ducts.
4. Ductular proliferations.
5. Alterations of the perisinusoidal space (sinusoidal capillarization).
6. Portal tract cellular infiltration.
7. Cholestasis.
8. Fatty change of hepatocytes.

The detection of these lesions was based on definitions given in the literature (Weinbren et al., 1985; Portmann et al., 1985; MacSween et al., 1987; Baptista et al., 1988). For severity grading of lesions, a system previously proposed and extended for the purpose of the present analysis was employed (Schweizer et al., submitted). Scoring of inflammatory changes, of piecemeal necroses and of fibrotic changes was based on histology activity indices previously described (Knodell et al., 1981; Lindh et al., 1988), with some modifications.

As emphasized above, morphological criteria were selected according to the working strategy that 1) lesions to be analyzed should be amenable to reproducible assessment based on routine sections, and 2) histological parameters should cover both resident and migratory cells of the liver, and the hepatic extracellular matrix. Ito cells were not assessed, as this cell type could not be reproducibly identified in our routine archival material. In contrast, littoral cells with a dendritic morphology and being immunoreactive for smooth muscle actin were registered and were, for the purpose of this study, accepted as being myofibroblast-like cells probably derived from Ito cells.

For each light microscopic parameter, the scoring criteria are listed in the *Appendix*.

Immunohistochemical parameters

Immunoreactivity for vimentin in ductular cells has previously been reported (Nakanuma and Kono, 1992; Rontogianni et al., 1994), and may be an indicator of immaturity of proliferating epithelial cells. Hence, it was of interest to test as to whether this phenomenon would also occur in ductular proliferations seen in AHC.

Expression of smooth muscle actin (SMA) was assessed in littoral and periportal cells with a dendritic morphology. These myofibroblast-like cells are currently thought to represent activation forms of Ito cells and to take a central position in hepatic fibrogenesis (Sztark et al., 1986; Weiner et al., 1990).

Proliferating cell nuclear antigen (PCNA/cyclin; Hall and Woods, 1990) was assessed as previously described (Hall et al., 1990; Gelb et al., 1992; Jankowski et al., 1992; Kawakita et al., 1992; Kayano et al., 1992; Koukoulis et al., 1992; Shresta et al., 1992). In normal livers, only very few hepatocyte nuclei show immunoreactivity for PCNA, and most positive nuclei are found in the littoral cell population (Koukoulis et al., 1992). In the present study, the PC 10 monoclonal mouse anti-PCNA antibody was used (DAKO-PCNA PC 10) which has recently been shown to stain biopsies more reliably than 19A2 (Gelb et al., 1992). The tissue samples analyzed had been fixed in formalin for less than 30 hours which is important because both the choice of fixative and the duration of fixation may affect the accessibility of PCNA/cyclin antigen to antibody detection (Bravo and MacDonald-Bravo, 1987; Galand and Degraef, 1989; Garcia et al., 1989; Gelb et al., 1992). Nucleated cells were considered positive for PCNA only if definite bright staining of the nucleus was identified (Hall et al., 1990; Yu et al., 1991; Koukoulis et al., 1992). PCNA labelling was indicated as mean numbers of positive nuclei per 20 high power fields (HPFs, i.e. at x 400 magnification).

Immunoreactivity for the major basement membrane component, collagen IV, was assessed in the perisinusoidal space in order to obtain a measure of sinusoidal capillarization. Under normal conditions, the perisinusoidal space contains no or only discontinuous basement membranes (Rappaport, 1973), whereas basement membrane is deposited in conditions resulting in sinusoidal capillarization (Rojkind and Dunn, 1979).

Immunoreactivity for factor VIII-related antigen (FVIII-Ag) was assessed in vascular and sinusoidal endothelia. The reason why we attempted to test immunoreactivity for FVIII-Ag was twofold. Firstly, we were interested in analyzing as to whether sinusoidal capillarization would be associated with the appearance of FVIII-Ag in sinusoidal endothelia; and secondly, we aimed at testing whether biliary piecemeal necroses as potential starting sites of septa would contain vessels expressing FVIII-Ag. The protocol used for this part of the study is given in the *Appendix*.

Results

Light microscopy and immunohistochemistry

Varying degrees of fibrosis were seen in all (16/16) samples obtained from grossly atrophic areas. Table 3 shows that fibrosis was of the septal type (incomplete or complete, i.e. portoportal septa) in the majority of cases, and that fibrotic changes were associated with nodular change in 13/16 cases (81.2%), in part corresponding to a change frequently termed, «secondary biliary cirrhosis» (Fig. 1). The question whether this term is appropriate will be discussed below. In contrast, only 2/4 samples from hypertrophic areas showed fibrosis, and it was of low degree (grades 1 and 2; Table 3).

Biliary piecemeal necrosis (BPMN; Fig. 2) was present in 16/16 atrophic areas, and it was severe (grade 4) in almost a third of the samples (Table 3). All samples with grade 4 BPMN also showed advanced fibrosis with nodular change of parenchyma. BPMN was also noted in hypertrophic regions, but it was of low degree throughout (2/4 grade 1, 2/4 grade 2).

Alterations of interlobular and septal bile ducts were encountered in 14/16 cases with atrophy, characterized in 9/16 by either ductal necrosis or even ductal

destruction (Table 4). Minor lesions (vacuolar change of epithelia and/or disarray of epithelial nuclei) were found in 3/4 cases with hypertrophy (Table 4). Ductular proliferations were lacking in only 1/16 cases with atrophy. They were marked in 11/16 (68.7%), and samples showing the most advanced proliferation were those with severe septal fibrosis and nodular change (6/6%; Table 4). It was of interest to note that slight degrees of ductular proliferation also occurred in hypertrophy (3/4, with grade 1 and 2 lesions), even in the absence of septal fibrosis. When comparing the scores obtained with conventional preparations with those based on cytokeratin staining it turned out that 76.5% of findings seen in H&E-stained preparations were supported by immunohistochemistry, and in 4 samples only the results were not in line. The higher sensitivity of immunostaining of ductular structures resulted in a higher grading in 3/4 of these samples (Table 4, Figs. 3A,B). For atrophy, 9/13 samples analyzed exhibited ductules with immunoreactivity for the intermediate filament, vimentin, and only 4 samples were clearly negative, whereas 3/3 samples from hypertrophic parts showed few vimentin-positive ductules (grade 1; Table 4). It turned out that positivity for vimentin was found predominantly in those situations where ductular proliferation was more developed (77.7% in the atrophic parts).

Alterations of the perisinusoidal space of Disse were noted in both atrophic and hypertrophic areas, but to a different extent. Collagen IV immunoreactivity along Disse's space (Fig. 4) covering more than 75% of the surface analyzed was detectable in 42.8% in atrophy, and in one third in hypertrophy (Table 5). It was striking to note that grades 2 and more were seen in all three cases of hypertrophy. The observation that changes in the perisinusoidal compartment may also occur in hypertrophy is further supported by the observation that spindle cells immunoreactive for SMA were found in a littoral compartment in all samples from hypertrophic parts, and they were also present or increased in number in all sections from atrophic areas (Table 5), sometimes clustered in BPMN, at the limiting plate of parenchyma, or around ductular proliferations. However, high densities (grade 3) were chiefly found in atrophy (40%)

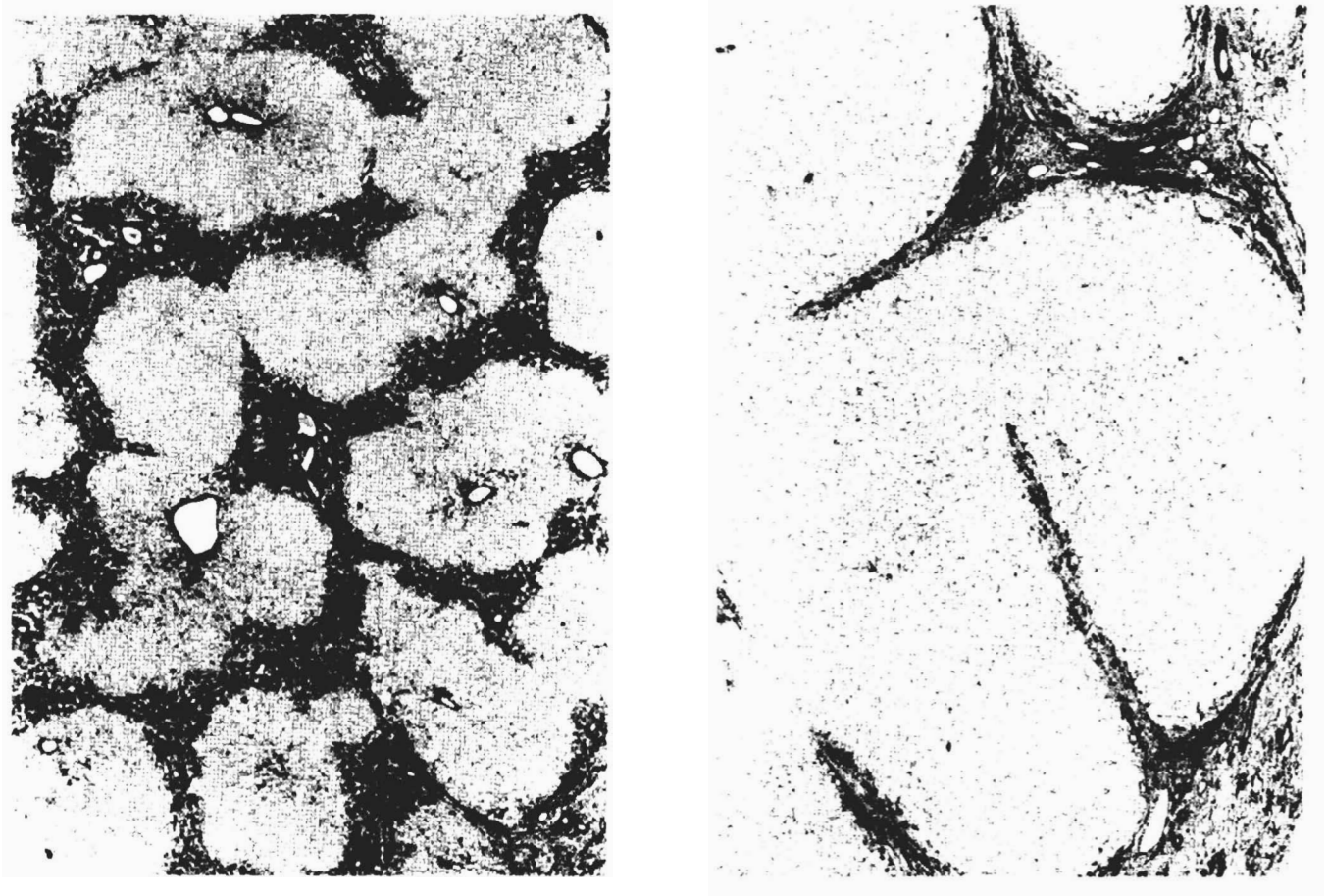

Fig. 1. A. Liver atrophy with formation of complete, porto-portal septa, portal tract fibrosis, increase of small bile duct/ductule number, and partial nodular change. Even though the overall aspect is reminiscent of liver cirrhosis, the vascular relationships are partially preserved. Type IV collagen immunostain. x 25. **B.** Liver atrophy due to benign bile duct stenosis. Lobules are grossly deformed and asymmetrical, and a nodular aspect ensues, in particular to the left hand side of the figure. This configuration, however, does not correspond to true cirrhosis. Type IV collagen immunostain. x 50

with fibrosis and nodular change, and focal accumulations of SMA-positive myofibroblasts were a dominant feature in this group (93.3%; Table 5).

A particularly interesting result was obtained when testing immunoreactivity for FVIII-Ag. In atrophy, 53.8% of cases showed no positivity of endothelia located within liver lobules or nodules, whereas positivity was grade 1 or 2 in 30.9%, and grade 3 in 15.3%. In contrast, no sinusoidal reactivity for FVIII-Ag was observed in hypertrophy (Table 6). Furthermore,

Table 3. Grading of liver fibrosis and biliary piecemeal necrosis (BPMN).

	FIBROSIS							BPMN					
GRADES	0	1	2	3	4	5	6	0	1	2	3	4	5
Atrophy													
1					X					X			
2					X							X	
3						X				X			
4				X						X			
5						X				X			
6					X						X		
7				X						X			
8		X								X			
9			X						X				
10					X							X	
11				X					X				
12					X							X	
13						X						X	
14		X							X				
15					X				X				
16					X							X	
Hypertrophy													
1			X							X			
2		X							X				
3	X									X			
4	X								X				

Criteria for grading: see Appendix

Table 4. Grading of bile duct lesions, ductular proliferations, and vimentin immunoreactivity in ductular epithelia.

	BILE DUCT LESIONS				DUCTULAR PROLIFERATION					VIMENTIN IN DUCTULES			
GRADES	0	1	2	3	0	1	2	3	4	0	1	2	3
Atrophy													
1	X						X						
2			X					X(X)			X		
3				X		X(X)						X	
4		X				X	(X)			X			
5			X						X(X)			X	
6			X						X(X)		X		
7		X					X(X)				X		
8	X				(X)	X							
9		X					X(X)			X			
10			X						X(X)				X
11			X				X(X)			X			
12			X						X(X)		X		
13			X						X(X)			X	
14		X				X(X)				X			
15				X	X								
16		X				X(X)					X		
Hypertrophy													
1	X						X						
2		X				X		(X)			X		
3	X					X(X)					X		
4	X					X	(X)				X		

Criteria for grading: see Appendix. (X) in ductular proliferations: results obtained with cytokeratin 19 immunostaining. For atrophy cases 1, 8 and 15 and for hypertrophy case 1, vimentin immunostaining could not be performed.

FVIII-Ag-positive structures were found in 61.5% of BPMNs registered in atrophy, indicating that BPMNs contain endothelial cells with an immunophenotype different from that of normal sinusoidal endothelia, and that vascularisation may start at sites where BPMNs ensue. FVIII-Ag-positive cells in BPMN were also noted in one sample with hypertrophy (Table 6).

Testing for PCNA immunoreactivity revealed that a moderate or marked increase in PCNA staining of hepatocyte nuclei was seen in 12/14 cases with atrophy. Thus, proliferative activity occurred also in areas expected to be in proliferative quiescence, but the

Table 5. Grading of immunoreactivity for collagen IV and smooth muscle actin (SMA)

	COLLAGEN IV					SMA IN MYOFIBROBLASTS (overall cellularity)			
GRADE	0	1	2	3	4	0	1	2	3
Atrophy									
2				X					X
3				X			X		
4					X		X		
5					X				X
6					X				X
7	X						X		
8		X					X		
9				X			X		
10					X				X
11				X				X	
12	X							X	
13					X				X
14		X					X		
15								X	
16					X				X
Hypertrophy									
2					X		X		
3				X			X		
4			X						X

Criteria for grading: see Appendix. Collagen IV could not be assessed in cases 1 and 15 (atrophy) and in case 1 (hypertrophy). SMA immunostaining could not be performed in case 1 of both atrophy and hypertrophy groups.

Table 6. Grading of immunoreactivity for FVIII-Ag.

	INTRALOBULAR AND INTRANODULAR LITTORAL CELLS				POSITIVE CELLS IN BPMNs			
GRADE	0	1	2	3	0	1	2	3
Atrophy								
3 focal				X				X
diffuse			X					
4	X				X			
5	X					X		
6	X				X			
7 focal			X				X	
diffuse		X						
8	X				X			
9 focal			X			X		
diffuse		X						
10 focal				X				X
diffuse			X					
11	X				X			
12 focal			X				X	
diffuse		X						
13 focal			X					X
diffuse		X						
14	X				X			
16 focal		X					X	
diffuse	X							
Hypertrophy								
2	X					X		
3	X				X			
4	X				X			

Criteria for grading: see Appendix. No immunostaining was possible in cases 1, 2 and 15 (atrophy) and case 1 (hypertrophy).

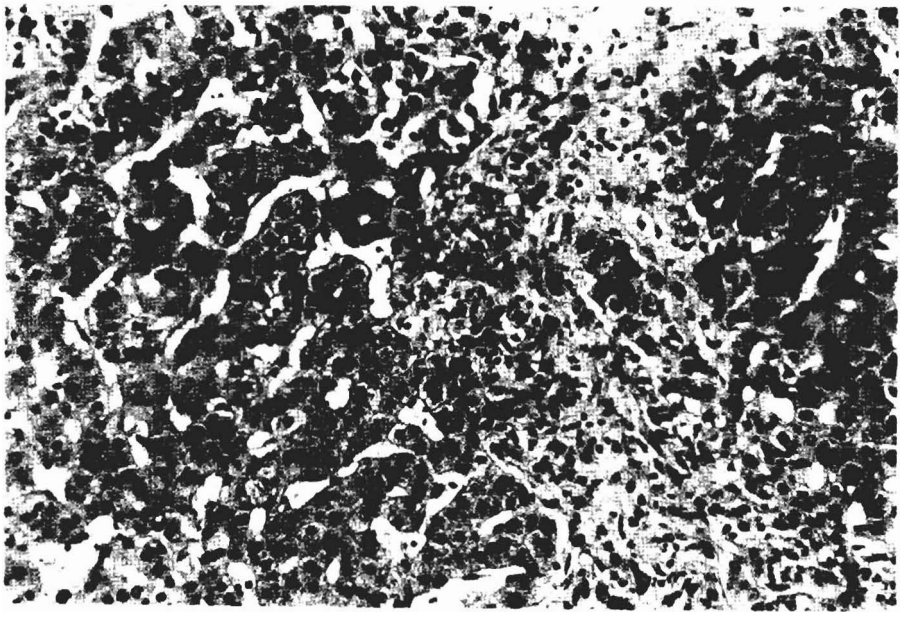

Fig. 2. Liver atrophy due to benign bile duct stenosis. The portal tract is oversized and fibrotic. Note that the limiting plate is focally interrupted (to the left of the middle) by a fibrous tissue containing round cell infiltrates and ductular proliferations. This interface lesion corresponds to so-called biliary piecemeal necrosis. Haematoxylin-eosin stain. x 150

estimated number of PCNA-positive hepatocyte nuclei was higher in the hypetrophic samples (Table 7).

Inflammatory infiltrates of varying intensity were noted in all cases of atrophy. They mostly consisted of lymphocytes and their predominant localization was at the portal tracts (PT), where they involved less than one third of PT in 18.7%, one to two thirds of PT in 37.5%, and more than two thirds in 43.7%. Dense infiltrates involving up to two thirds of PT were, however, also found in hypertrophic areas (2/4). Overall, atrophy and hypertrophy were different with respect to PT infiltration by the density of infiltrates only, but not by the type of cells or their spatial distribution.

Morphological signs of cholestasis were encountered in 56.2% of samples with atrophy; in 2/16 it was grade 1 (grading: see *Appendix*); in 5/16, grades 2 or 3, and in 2/16, grade 4. But also hypertrophic areas may show this change, in that grade 1 cholestasis was found in one case, and a grade 2 or even 3 lesion in another.

In the present material, fatty change of hepatocytes was seen in both atrophic and hypertrophic liver regions. In atrophy, it was marked (i.e., involving 20% or more of the section surface) in only 2/16 cases, and moderate in a further four samples. A low degree fatty change was seen in 1/4 samples with hypertrophy.

Light microscopic and immunohistochemical features as a function of underlying disease

In a second step we were interested in analyzing as to whether frequences and intensities of lesions found in either atrophy or hypertrophy were related to the type of disease inducing AHC. For this purpose, we divided the material into two groups allocated either to longstanding obstruction due to benign stenosis or stricture (BOD), or to rapidly evolving stenosis due to malignant neoplastic disease (MOD). For atrophy, it is striking to note that marked septal fibrosis associated with nodular change of parenchyma was found in 100% of BOD, but only in 40% of MOD. Slight degrees of fibrosis seen in hypertrophy were all in cases with BOD. A similar pattern ensued for BPMN,

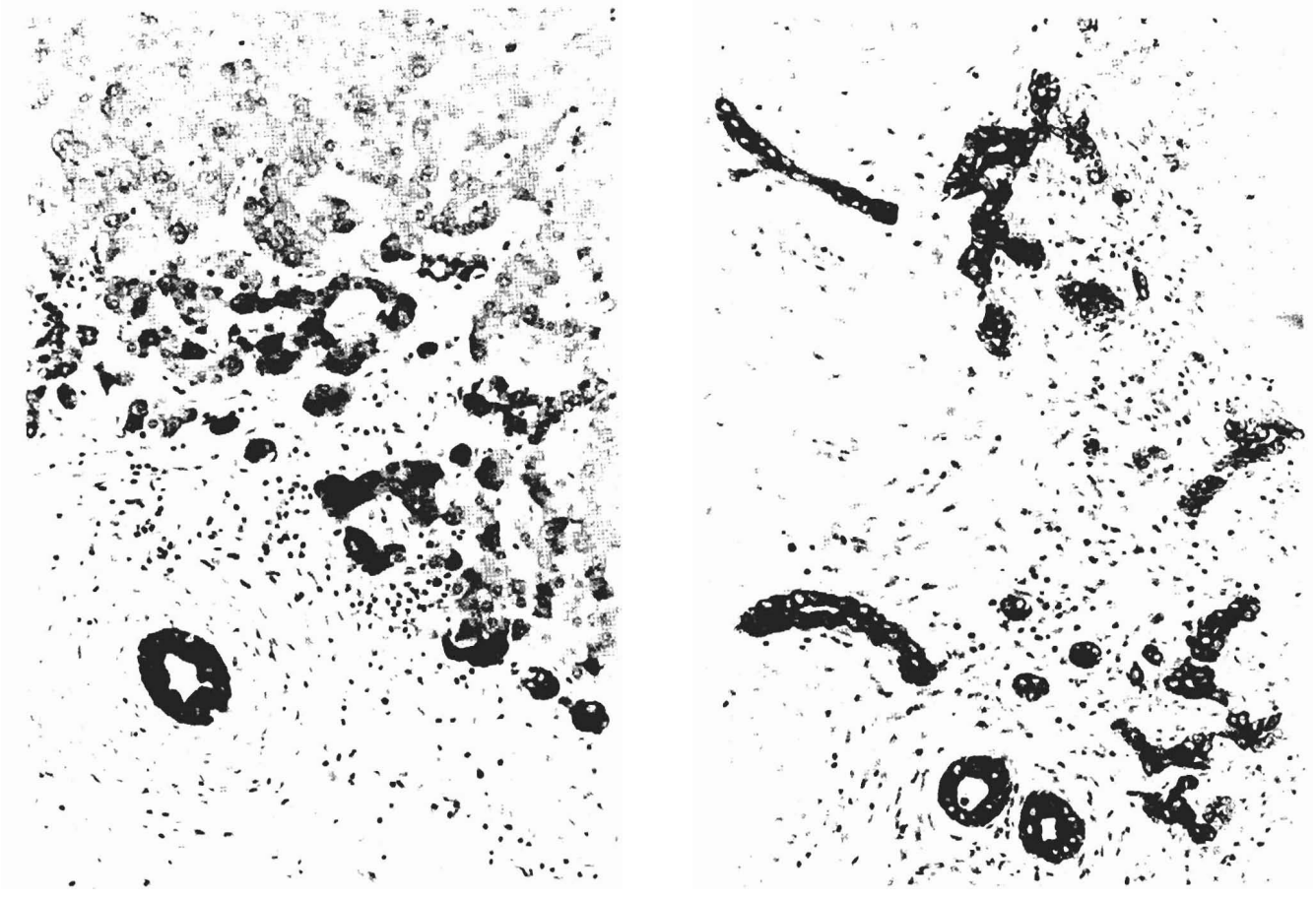

Fig. 3. A. Liver atrophy. In this cytokeratin 19 immunostain, a triangular gap in peripheral parts of lobular zone 1 is clearly visualized (biliary piecemeal necrosis). Immunoreactive single cells or cell clusters represent elements of ductular proliferations, which cannot easily be discerned in a routine preparation. The interlobular bile duct exhibits slight periductal fibrosis. Cytokeratin 19 immunostain. x 200. **B.** In a more advanced stage, ductular proliferations embedded in portal tract fibrous tissue are clearly seen. Cytokeratin 19 immunostain. x 150

where 31% of severe BPMN were in the BOD, and 20% in the MOD group, respec-tively, and for bile duct changes, where high-degree lesions in atrophy were 72.7% due to BOD, and only 20% due to MOD. Higher degrees of ductular prolifera-tion seem to be more frequent in atrophy associated with BOD. Sinusoidal capillarization also predominates in the BOD situation, in that 90% of these cases had signs ofcapillarization of at least grade 2, whereas 75% of MOD cases had a lesion less than grade 3.

Table 7. PCNA labelling of hepatocyte nuclei

	GRADES			
	0	1	2	3
Atrophy				
2			X(13.74)	
3			X(11.50)	
4		X(4.75)		
5				X(20.65)
6				X(25.55)
7			X(9.50)	
8				X(31)
9				X(29)
10			X(8.25)	
11		X(3.75)		
12				X(29.60)
13				X(16.60)
14			X(13.10)	
16			X(14.30)	
Hypertrophy				
2			X(12.15)	
3				X(32.15)
4			X(8.50)	

Figures in brackets: mean value of counts per 20 HPFs. Cases 1 and 15 and case 1 for groups with atrophy and hypertrophy, respectively, could not be analyzed.

Discussion

AHC of the liver, the clinical relevance of which has only recently been recognized, is an important hepatic pathology with a wide range of underlying diseases (Benz et al., 1952; Leevy et al., 1954; Schalm et al., 1956; Barbier and Van der Stricht, 1969; Christofferson and Poulsen, 1970; Gautier-Benoit and Houcke, 1973; Ham, 1974, 1990; Myracle et al., 1981; Czerniak et al., 1986; Hadjis et al., 1986a,b, 1989; Hadjis and Blumgart, 1987, 1988; Rozga et al., 1988; Mohd et al., 1989; Ruf et al., 1989; Schweizer et al., 1992). Its increasing significance relates to new possibilities in surgery. In fact, AHC represents a complex hepatobiliary disorder which is frequently due to mechanical obstruction of the biliary and/or vascular structures and needs, in many instances, surgical management. However, the diagnostic approach has to be appropriate, and a multidisciplinary strategy should be employed in order to arrive at a precise preoperative diagnosis (Schweizer et al., submitted). Pre-operative planning does not only include diagnosis of AHC as such, but also calls for the identification of those subsets of patients which may profit best from a surgical intervention. Thus, questions arising with respect to the pathology of AHC are threefold. Firstly, we ought to known whether atrophy can clearly be distinguished from hypertrophy based on biopsy samples and, provided this is the case, if a routine methodology would suffice to achieve this goal. Secondly, we have to analyze if in AHC, and particularly in the atrophic component, lesion patterns may occur having a degree of severity rendering a successful recovery after intervention less probable. And, thirdly, one should know whether hepatic alterations in AHC would differ with respect to the type of underlying disease, in particular benign versus malignant

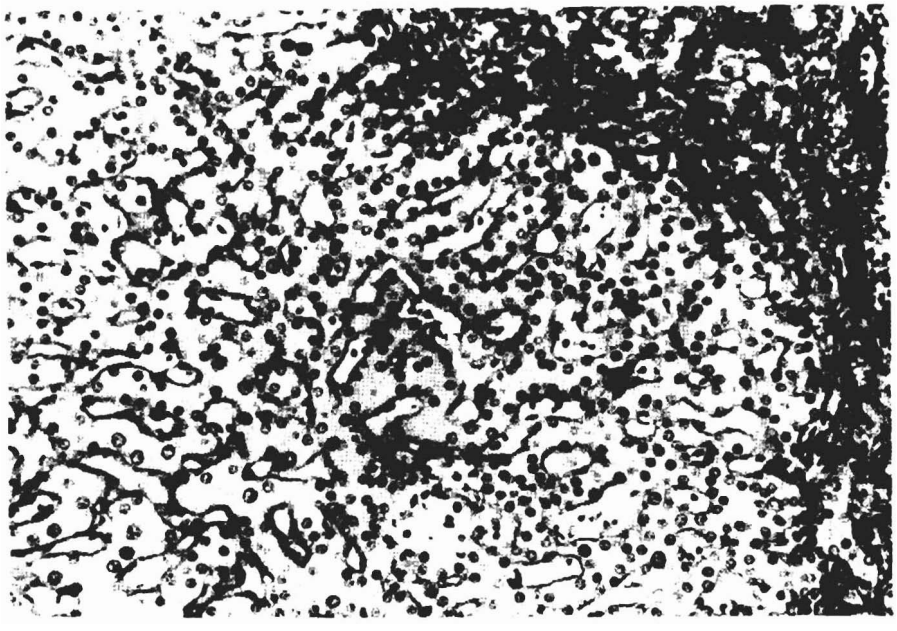

Fig. 4. Liver atrophy with deformation of a lobule and formation of fibrous septa. In this type IV collagen immunostain, part of the sinusoids are shown to be lined by a dark reaction product, indicating collagen IV deposition and basement membrane formation, and representing sinusoidal capillarization. Type IV collagen immunostain. x 150

obstruction. In order to approach this set of questions, the present histopathological investigation has been undertaken, based on a group of patients with well-documented AHC.

An outstanding histological feature observed in liver tissue obtained from macroscopically atrophic areas is portal tract-associated septal fibrosis, as has been emphasized before (Weinbren et al., 1985). Fibrosis of hepatic tissue was found in all of the cases analyzed in the present study, and it was severe in the majority of samples, i.e. with formation of complete septa. Advanced fibrosis was associated with nodular change of parenchyma in about 80% of cases, but with preservation of vascular relations. Thus, even though samples with the most severe derangement of overall architecture corresponded to a histological picture termed, «biliary cirrhosis», formation of true pseudoacini representing a hallmark of cirrhosis in the proper sense of this term was not found. This observation is in line with former work stressing that even in longstanding and severe biliary obstruction true «biliary cirrhosis» may rarely, if ever, ensue (Johnstone and Lee, 1976). Nodules, as seen in severe atrophy, may thus represent some sort of parenchymal remodelling in concert with septal fibrosis rather than an irreversible change characterized by pseudoacini with loss of normal vascular relationships. Hence, biliary fibrosis with nodular change may be a term more appropriate than «biliary cirrhosis» to denote this particular lesion pattern. This view is supported by the recent finding that cirrhosis-like changes experimentally produced in rats through bile duct ligation may be reversible upon later correction via Roux-en-Y anastomosis (Zimmermann et al., 1992).

The pathogenesis of fibrosis occurring in liver atrophy and, albeit to a much lesser degree, in hypertrophy, is not yet clear (MacSween and Burt, 1989). The present investigation uncovered, however, a lesion with a dominant expression mainly in atrophy and possibly involved in the formation of fibrous septa, i.e. biliary piecemeal necrosis (BPMN; Portman et al., 1985). Piecemeal necroses are a well-known hallmark in chronic active viral hepatitis and are morphologically characterized by severing of the lobular limiting plate associated with cellular infiltration and followed by collagen deposition (so-called interface lesions in modern parlance). PMNs are known to occur in chronic diseases of the biliary tract, including primary biliary cirrhosis (Rontogianni et al., 1994), and are termed BPMN in these situations (Portmann et al., 1985). The differential diagnosis of BPMN and other forms of periportal liver injury and inflammation has recently been reviewed by an international group (Baptista et al., 1988). To the best of our knowledge, BPMN has not been systematically studied in cases of AHC. In the present analysis we noted that BPMN was present in all cases with atrophy, and it was severe in almost a third, whereas it was of low degree in hypertrophy throughout. The fact that severe BPMN in atrophy was observed in those situations where advanced septal fibrosis with or without nodular change of parenchyma was also found may indicate that there is a relationship between BPMN, which is a florid lesion, and the formation of fibrous septa. This hypothesis has formerly been suggested for septum formation in PBC (Nakanuma, 1991) and is supported by the present finding that areas involved with BPMN are frequently vascularized in liver atrophy, in that almost two thirds of such lesions contained cells immunoreactive for FVIII-Ag. BPMNs may theoretically be induced by infection (e.g., cholangitis), by hepatocyte damage in the lobular periphery due to cholestasis (Calmus et al., 1990, 1992), or by an autoimmune reaction following bile duct, ductular or hepatocyte damage. In fact, bile duct damage, in part resulting in necrosis or even duct destruction, was found in a majority of samples with atrophy, and the lesions were not of the purulent infectious cholangitis type. We may thus assume that biliary obstruction can induce, in addition to infection, a complex pattern of alterations of bile duct and ductular epithelia, which may also later involve the limiting plate and the lobular periphery. A mechanism of this type has been suggested to play a pathogenic role in the evolution of AHC-like changes occurring in PBC (Rontogianni et al., 1994). An additional factor, which may represent a driving force in BPMN formation, is ductular proliferation. Ductular proliferations are a well-known phenomenon in a wide array of chronic hepatic diseases, but they are particularly prominent in obstructive biliary disorders (Desmet, 1987). The mechanisms leading to an increase in ductule number are not known so far, even though promising approaches for a better understanding of their pathogenesis have recently been published (Zhao et al., 1993a). In atrophy of the liver, ductular proliferation was marked in more than two thirds of the cases, and the immunoreactivity of their epithelia for vimentin may indicate a certain degree of immaturity of these structures. Vimentin-positivity of ductules has formerly been reported for two other situations of hepatic disease, including PBC (Nakanuma and Kono, 1992; Rontogianni et al., 1994). Interestingly, high degrees of ductular proliferation in atrophy were associated both with severe BPMN and advanced septal fibrosis. The relationship between proliferated ductules and BPMN as a potential precursor lesion of fibrous septa is not known, but transformation of perisinusoidal Ito cells into myofibroblasts has been observed in areas of ductular proliferation (Callea et al., 1982; Callea and Desmet, 1985). In addition, it has recently been demonstrated that hepatic myofibroblasts, which are the collagen-producing offspring of Ito cells (Callea and Desmet, 1985), do not only make part of the littoral (perisinusoidal) subset of hepatic cells, but may also form an extralittoral compartment located around hepatic artery branches, bile ducts, and ductules (Zhao et al., 1993b). In the present study, myofibroblast-like cells expressing smooth muscle actin were frequently clustered at the limiting plate, around ductules, and in BPMNs. Based on

these findings it is tempting to assume that collagen deposition in BPMNs as a starting point for septum formation may derive from a periductular and periarterial compartment of resident myofibroblasts (MFBs). MFBs were also noted in larger amounts in the perisinusoidal space in atrophy, and to a lesser degree in hypertrophy. An increased number of this cell type, which appears to be the major cell involved in the formation of the perisinusoidal extracellular matrix, is of importance in the light of a further finding morphologically characterizing liver atrophy, i.e. capillarization of sinusoids. Based on the fact that, under normal conditions, the perisinusoidal space contains no or only discontinuous basement membranes, the finding of a strong immunoreactivity for the major basement membrane component, collagen type IV, in large areas of liver atrophy is a strong indicator that in fact capillarization has taken place in this situation. This is further underlined by our observation that sinusoidal endothelia become immunoreactive for FVIII-Ag. The sinusoids of normal liver are lined by unique type of endothelial cell differing from vascular endothelial cells in the absence of an underlying basement membrane and their apparent failure to express FVIII-Ag (Irving et al., 1984; Wisse et al., 1985; Nagura et al., 1986). In the human, FVIII-Ag was localized throughout the vascular and sinusoidal compartments in some studies (Hoyer et al., 1973; Koike and Matsuoka, 1986; Kwast et al., 1986), but not in others (Fortwengler et al., 1981; Holthofer et al., 1982; Wion et al., 1985). In a recent rodent study, FVIII-Ag immunoreactivity was limited to larger hepatic vessels, and no or minimal sinusoidal endothelial staining was detected regardless of the techniques employed (Lenzi et al., 1990). The lack of expression of FVIII-Ag in sinusoidal endothelia may be related to the poorly developed or lack of extracellular matrix at this site, since FVIII-Ag is also stored in the subendothelium (Rand et al., 1980). It has been suggested that during the development of chronic liver disease, sinusoidal endothelial cells undergo transformation to a vascular-type endothelium, a phenomenon which is thought to have a strong influence on the maintenance of hepatic function (Hahn et al., 1980; Bianchi et al., 1984; Petrovic et al., 1989). Thus, the development of FVIII-Ag-positive sinusoidal endothelia in lobules or nodules is in keeping with the concept of capillarization of sinusoids originally proposed by Schaffner and Popper (1963). A similar phenomenon has recently been described in primary biliary cirrhosis (Babbs et al., 1990). Even though cholestasis was more pronounced in atrophy as compared to hypertrophy, this parameter may not be a useful discriminator. Even less or not at all helpful were cellular infiltrates, fatty change of hepatocytes, and the proliferative activity of hepatocytes as based on PCNA immunostaining. The fact that cholestasis was also seen in the hypertrophic parts is worth noting, because it may indicate that, via a mechanism unknown so far, a derangement of bile clearance may involve hepatic regions not directly hit by the lesions producing atrophy in the other parts. That PCNA labelling of hepatocytes may be increased in hypertrophic areas is not unexpected, because it has been demonstrated that hypertrophy of the liver is accompanied by hyperplastic changes leading, among others, to triplicate hepatic cords (Weinbren and Hadjis, 1990). In contrast, a corresponding increase of proliferative activity in the atrophic part has not been described so far, and is not easy to explain, because current theory would predict that cell proliferation should be decreased in tissues undergoing atrophy or involution. However, atrophy may in fact represent a dynamic process rather than a static end-point according to traditional views, and increased proliferative activity may just be an indicator of active tissue remodelling at sites of BPMNs and in parenchymal nodules occurring in AHC.

Taken together, the following morphological features are highly characteristic for liver atrophy: 1) advanced septal fibrosis, frequently associated with nodular change of parenchyma sometimes resulting in a picture which has been reported as «biliary cirrhosis», but may preferably be termed, biliary fibrosis with nodular change; 2) biliary piecemeal necrosis (mostly severe), with formation of vascular sprouts and probably also representing preferred sites for the generation of fibrous septa; 3) ductular proliferations, frequently extending into septa and involving the parenchyma; 4) capillarization of sinusoids with deposition of collagen IV in the perisinusoidal space; 5) FVIII-Ag expression in sinusoidal endothelial cells; and 6) a seemingly paradoxical increase in proliferative activity of hepatocytes as based on PCNA staining. This typical constellation of findings not only allows a clear distinction between atrophic and hypertropohic parts, but also enables pathologists to predict liver atrophy in a biopsy specimen.

The severity of lesions observed in liver atrophy appears to be related to the type of the underlying disease. Thus, marked fibrosis with nodular change was much more frequent in benign obstructive bile duct disease (BOD) than in malignant stenosis (MOD), and slight fibrosis was even noted in the hypertrophic part in the case of BOD. A similar relationship was found for the parameters, BPMN, ductular proliferation, and sinusoidal capillarization. The reason for this difference between BOD and MOD is not yet clear, but may in principle be related to the usually longstanding obstructive situation in the former and the more rapid disease progression in the latter, resulting in an earlier intervention in MOD.

In a previous study on AHC (Schweizer et al., submitted) it has been shown that the overall severity grade of lesions in atrophy due to BOD is a good indicator for prognosis. Disorders with marked histological changes and benign underlying disease may provisionally be improved by surgery, but generally exhibit ongoing hepatic deterioration even after successful surgical de-compression. Conversely, patients

with BOD and less severe histological changes generally show good results and full recovery. Therefore, identification of atrophy and hypertrophy in AHC, and the definition of lesion severity are crucial elements in the adequate approach to this unique hepatic pathology.

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Appendix

Scoring criteria for histopathological and immunohistochemical parameters

1. Fibrosis
   - Grade 0: No fibrosis.
   - Grade 1: Slight fibrosis (portal tract fibrosis only).
   - Grade 2: Portal tract fibrosis plus incomplete fibrous septa.
   - Grade 3: Complete porto-portal septa.
   - Grade 4: Complete porto-central septa with incipient focal nodular change.
   - Grade 5: Diffuse incomplete or focally complete cirrhosis.
   - Grade 6: Diffuse complete cirrhosis.

2. Biliary Piecemeal Necrosis (BPMN)
   - Grade 0: No BPMN visible.
   - Grade 1: Minimal BPMN (one or few areas in single portal tracts).
   - Grade 2: BPMNs involve less than 50% of circumference of most portal tracts.
   - Grade 3: BPMNs involve more than 50% of circumference of most portal tracts.
   - Grade 4: As with grade 3, plus septal BPMNs.
   - Grade 5: As with grade 4, plus porto-portal bridging necroses.

3. Alterations of interlobular and septal bile ducts.
   - Grade 0: No changes.
   - Grade 1: Few ducts with vacuolization of epithelia and/or irregular arrangement of nuclei.
   - Grade 2: Focal epithelial necrosis of ducts.
   - Grade 3: Complete destruction of duct segments.

4. Ductular proliferation (DPs).
   - Grade 0: No DPs.
   - Grade 1: Few DPs in a minority of portal tracts.
   - Grade 2: DPs easily detectable in most portal tracts.
   - Grade 3: As with grade 2, but DPs involve lobular parenchyma.
   - Grade 4: Numerous DPs involving lobular parenchyma plus fibrous septa.

5. PCNA immunoreactivity of hepatocyte nuclei.
   - Grade 0: No PCNA-positive nuclei.
   - Grade 1: Few PCNA-positive nuclei (1-5 nuclei per 20 HPFs*).
   - Grade 2: Moderate (6-15 positive nuclei per 20 HPFs).
   - Grade 3: Strong PCNA immunoreactivity (more than 15 positive nuclei per 20 HPFs).
   - * HPF= high power field, x 400 magnification.

6. Immunoreactivity for vimentin in bile duct/ductular epithelia.
   - Grade 0: No immunoreactivity.
   - Grade 1: Immunoreactivity in very few ductular epithelia.
   - Grade 2: Immunoreactivity seen in several ductular structures.
   - Grade 3: Immunoreactivity in numerous ductular structures.

7. Cellularity of SMA-positive myofibroblasts (MFBs).
   - Grade 0: No MFBs.
   - Grade 1: Few SMA-positive MFBs.
   - Grade 2: Moderate amount of SMA-positive MFBs.
   - Grade 3: Numerous SMA-positive MFBs.

8. Immunoreactivity for collagen IV (col IV) in perisinusoidal space.
   - Grade 0: No reaction product visible in 20 HPFs.
   - Grade 1: Immunoreactivity for col IV in up to 25% of surface in 20 HPFs.
   - Grade 2: Immunoreactivity for col IV in up to 50% of surface in 20 HPFs.
   - Grade 3: Immunoreactivity foe col IV in up tp 70% of surface in 20 HPFs.
   - Grade 4: Immunoreactivity for col IV in more than 75% of surface in 20 HPFs.

9. Intralobular/intranodular littoral cells immunoreactive for FVIII-Ag.
   - Grade 0: No immunoreactivity.
   - Grade 1: Few FVIII-Ag-positive littoral cells.
   - Grade 2: FVIII-Ag-positive littoral cells regularly seen at medium magnification.
   - Grade 3: FVIII-Ag-positive littoral cells in high density.

10. FVIII-Ag-positive cells in BPMNs.
   - Grade 0: No immunoreactivity.
   - Grade 1: Few FVIII-Ag-positive cells in few BPMNs.
   - Grade 2: Several BPMNs at the periphery of lobules or nodules showing FVIII-Ag-positive cells.
   - Grade 3: Periphery of lobules or nodules shows a mantle zone of densely placed BPMNs containing FVIII-Ag-positive cells.

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