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Comparative histology of acute hepatitis B and non-A, non-B in Leuven and Padova

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Summary. A histological study was performed on liver biopsies from patients with acute hepatitis A ($n=13$), B ($n=35$) and non-A, non-B (nAnB) ($n=35$) in search for microscopical features characteristic for each type of hepatitis. Biopsies from two centres (Padova, Italy and Leuven, Belgium) were studied in order to determine whether the histological pattern in acute hepatitis A, B and nAnB may differ from one centre to another. The histology of cases of hepatitis A and B from Italy and Belgium did not differ. Less liver cell plemorphism was found in hepatitis A than in B. Clear differences were observed between acute hepatitis nAnB occurring in Padova when compared with cases from Leuven. The Padova-biopsies obtained from patients with transfusion-induced viral hepatitis were mainly characterized by a high degree of lympho-histiocytic intrasinusoidal infiltration whereas the Leuven-biopsies, mostly taken in patients with sporadic hepatitis, were characterized by the presence of numerous acidophilic bodies and Mallory body-like cytoplasmic alterations. Morphologically, the latter cases appear to be closely related to hepatitis B.

Key words: Hepatitis – Viral – Human – Pathology

Introduction

Until now, a diagnosis of non-A, non-B (nAnB) viral hepatitis has had to be made by exclusion of known hepatotropic viruses. Pending the availability of reliable serological tests, this hepatitis may be considered to be

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caused by different agents. This may happen in the same geographical region but is even more likely in different areas. Several investigators have tried to find specific histological markers for acute nAnB hepatitis by comparing these cases with biopsies from acute hepatitis A and B in experimental animals (Popper et al. 1980) and in humans (Dienes et al. 1982; Kryger et al. 1982; Phillips and Poucell 1981; Scheuer et al. 1980). We studied the histological differences between acute hepatitis A, B and nAnB especially to find out whether the histological pattern in acute hepatitis B and nAnB differed from one centre to another.

Materials and methods

Materials

Liver biopsies showing a histopathological picture of acute hepatitis from two hospital centers (Leuven in Belgium and Padova in Italy) were studied. The number of cases, their aetiology, time of biopsy and origin (Padova or Leuven) are summarized in Table 1.

Hepatitis A was diagnosed when hepatitis A virus antibodies of the IgM type were positive in the serum. Hepatitis B was considered to be the cause of the disease when hepatitis B surface antigen (HBsAg) and antibodies to hepatitis B core antigen (HBcAg) were present in the serum. nAnB virus hepatitis in the Padova and Leuven cases was diagnosed in the absence of serological markers indicative for hepatitis B or hepatitis A infection. In all Padova cases a rise in the titer of anti-cytomegalovirus (CMV) antibodies or of anti-Epstein Barr-virus (EBV) antibodies were also excluded. All Padova cases had a history of open heart surgery with multiple blood- and/or blood products-transfusions (Realdi et al. 1982; Tremolada et al. 1983). In the Leuven cases serological exclusion of CMV or EBV was not done systematically. However, none of these biopsies showed the histology described for CMV-hepatitis (Vanstapel and Desmet, 1983). The histological aspects of mononucleosis infectiosa reviewed by Gowing (1975) were also absent in these cases. In only three Leuven patients a history of blood transfusion was present in possible relation to the acute nAnB hepatitis. The other patients were considered to be "sporadic" cases (Villarejos et al. 1975). In both the Leuven and Padova cases toxic liver damage was also ruled out historically.

All the biopsies from Padova were fixed in a 5% neutral formalin solution. The Leuven cases were routinely fixed in Bouin's solution. All biopsies were stained with haematoxylin and eosin, PAS after diastase digestion, Van Gieson and/or Gomori's reticulin stain. In most of the cases a stain for bilirubin and iron, and Shikata's orcein staining was also available.

Methods

Assessment of biopsies. All biopsies were separately studied by 2 of the authors without knowledge of the aetiology of the hepatitis or of the clinical evolution. Points of disagreement were re-evaluated together. The following variables were coded in a scale from 0 to 6 (absent to severe) in a semi-quantitative way.

1) Portal hepatitis: The density of the portal infiltrate was graded from 0 till 6; grade 6 was reserved for presence of lymph follicles. An attempt was made to analyse the nature of the portal inflammatory cell infiltrate. The same scale (0–6) was applied for the occurrence of lymphocytes, plasma cells, histiocytes, neutrophilic and eosinophilic polymorphonuclear cells.

2) Piecemeal necrosis: (PMN) (De Groote et al. 1968; Popper et al. 1965)

3) Bile duct lesion of the Poulsen Christoffersen type: (Poulsen and Christoffersen 1972)

4) Periportal fibrosis

5) Acidophil changes: under this heading were noted: Mallory body like alteration of the cytoplasm, acidophilic condensation of the whole cytoplasm and acidophilic bodies (Cavalli

Table 1. Presentation of all cases according to their final histological diagnosis, etiology, time of biopsy and origin (Padova or Leuven)

Histological diagnosis	Time of biopsy	Etiology					
		Hepatitis A		Hepatitis B		Hepatitis nAnB	
AHTC	<6 months	5	1 Padova 4 Leuven	23	7 Padova 16 Leuven	17	13 Padova 4 Leuven
	6–12 months	1	1 Leuven	6	6 Leuven	3	3 Leuven
AHTC/BL	<6 months	2	2 Leuven	4	2 Padova 2 Leuven	10	6 Padova 4 Leuven
	6–12 months	–		–		–	
AH	<6 months	4	1 Padova 3 Leuven	2	1 Padova 1 Leuven	5	3 Padova 2 Leuven
	6–12 months	1	1 Leuven	–		–	
Total	<6 months 6–12 months	13	2 Padova 11 Leuven	35	10 Padova 25 Leuven	35	22 Padova 13 Leuven

AHTC: acute hepatitis with signs of possible transition to chronicity; AHTC/BL: AHTC-borderline group; AH: classical acute hepatitis

et al. 1968; De Wolf-Peeters et al. 1981; Kerr et al. 1972) (Fig. 1a, 1b, 1c). For the latter, no distinction was made between classical acidophilic bodies and fragmented acidophilic bodies ("fragmented Councilman-like" bodies) (De Wolf-Peeters et al. 1981).

6) Ballooning (Ishak 1973, 1976)

7) Steatosis: This was subdivided in different types: micromediovesicular, macromediovesicular or lipid granulomas.

8) Ground glass hepatocytes

9) Cholestasis

10) Siderosis of hepatocytes and/or Kupffer cells

11) Diffuse intralobular infiltration of lymphocytes (Fig. 2a, b, c, d, e)

12) Diffuse intralobular infiltration of histiocytes (Fig. 2a, b, c, d, e)

13) Pigmented macrophages

14) Lytic necrosis: This was not graded semi quantitatively between 0 and 6, but according to its extent as focal, zonal and panlobular necrosis. Bridging necrosis was recorded as fresh uncollapsed porto-central or porto-portal areas of necrosis (N) or as already formed passive septa (S): These findings were registered as "present" or "absent".

According to the absence or presence of PMN in acute hepatitis, each biopsy was diagnosed either as classical acute hepatitis (fully developed or early declining phase) or as acute hepatitis with signs of possible transition to chronicity (AHTC) (Bianchi et al. 1971, 1977) (Fig. 2a). In the last group the degree of PMN was variable and a subgroup with a minimal degree of PMN in one or more of the portal tracts was classified as borderline AHTC (Vanstapel et al. 1983). Table 1 shows the frequency of this histological diagnosis in each of the different aetiological groups. The grouping in classical acute hepatitis, AHTC and borderline AHTC is important for planned follow-up studies on progression to chronic hepatitis (Vanstapel et al. 1983); it also serves to emphasize the variability of the histological picture within the same aetiological group of acute hepatitis. For the purpose of the present study, however,

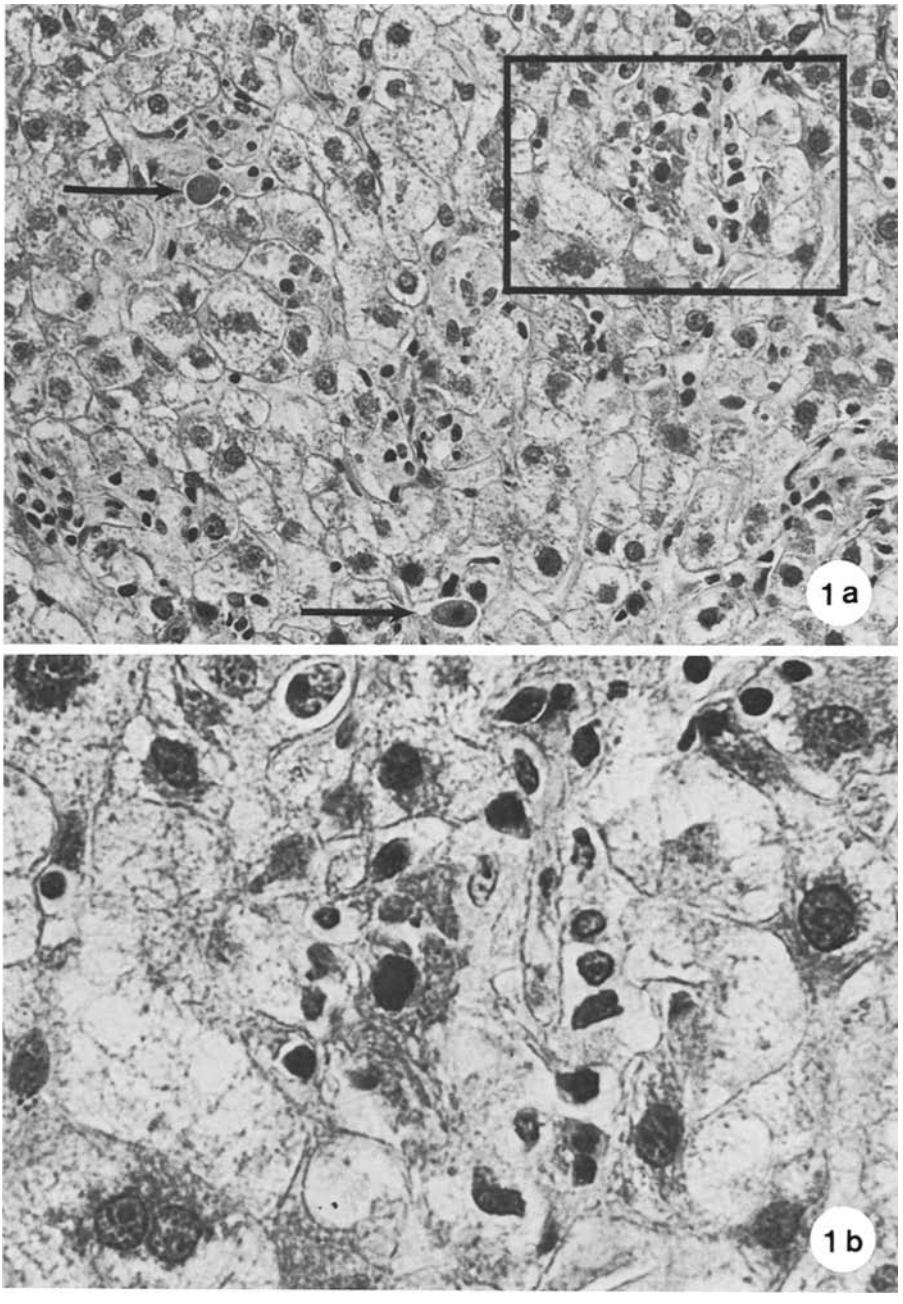
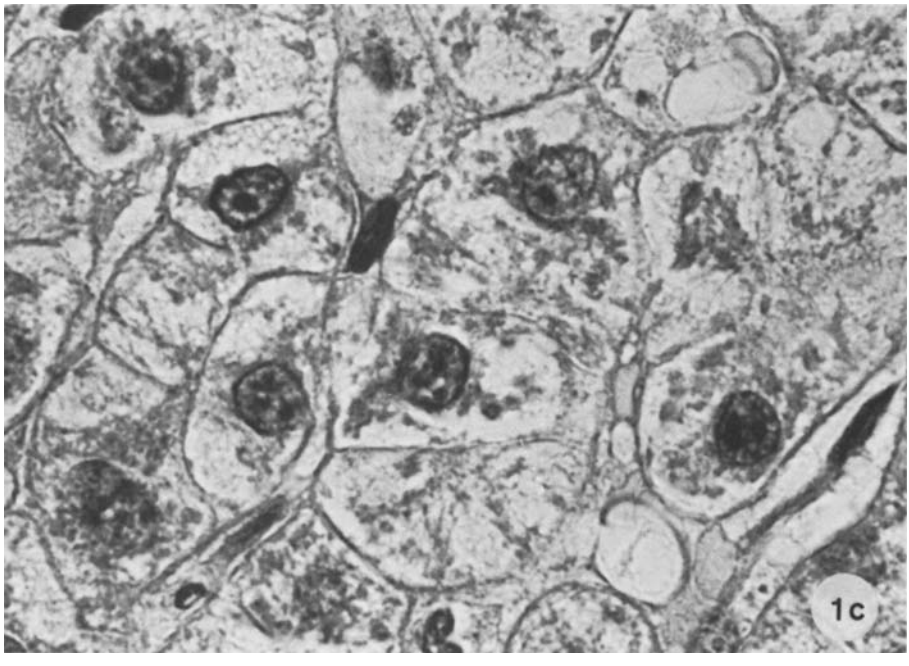


Fig. 1a–c. Hepatitis B (Leuven). **a** Focal areas of intralobular lymphohistiocytic infiltration and classical acidophilic bodies (*arrows*) are seen together with fragmented "Councilman-like bodies" (setting). This degree of Councilman (acidophilic) bodies was graded on scale 6. (HE ×320). **b** A higher magnification of the setting in (**a**) showing more detail of a fragmented "Councilman body like" structure: the cytoplasm is broken up in small eosinophilic granular fragments and the nucleus becomes pycknotic. Some lymphocytes are scattered around this focus. (HE ×920). **c** Hepatitis nAnB (Leuven). Coarse granular cytoplasmic condensations mostly in the perinuclear area as present in the majority of these hepatocytes are called Mallory body-like cytoplasmic alterations. This degree of Mallory body-like alteration was graded on scale 6. (HE ×1,600)



all variants of acute hepatitis were grouped together to compare the aetiological types A, B and nAnB.

Statistical methods. For each morphological criterion, the data were cast in a two-way table (Table 2a, b). The rows represent the various aetiological categories, and the colums represent the scale on which the morphological features are measured. Mostly the values were ranked on an ordinal scale, extending over 7 values, from 0:absent, to 6:present in its most obvious form. A few mutually exclusive features were classified on a nominal scale. The number of cases in each cell are given. Nonparametric statistical methods were used (Sidney, Siegel 1956).

The Chi-square test was used to determine the significance of the differences among the 4 groups, for each morphological criterion. When necessary adjacent colums were combined so that fewer than 20% of the cells had an expected number of cases of less than 5 and no cell equals zero. Probability levels for Chi-square were compared with the 5% level of significance. The different aetiological categories were combined two by two (Fig. 3) as independent samples. They were compared by the Fisher test, and one-sided exact probabilities were given. For each of the cases all the morphological features were coded. The resulting vectors were subject to cluster analysis.

Results

The frequency of each histological variable in all biopsies is summarized in Table 2a, b. A Chi-square test over all the aetiological groups revealed that no morphological criterion showed significant differences. We have therefore concluded that the variables studied are a poor tool for the differential diagnosis between the aetiological groups.

Subsequently we have examined the groups two by two thereby looking for the larger difference among the four groups. We know that such a

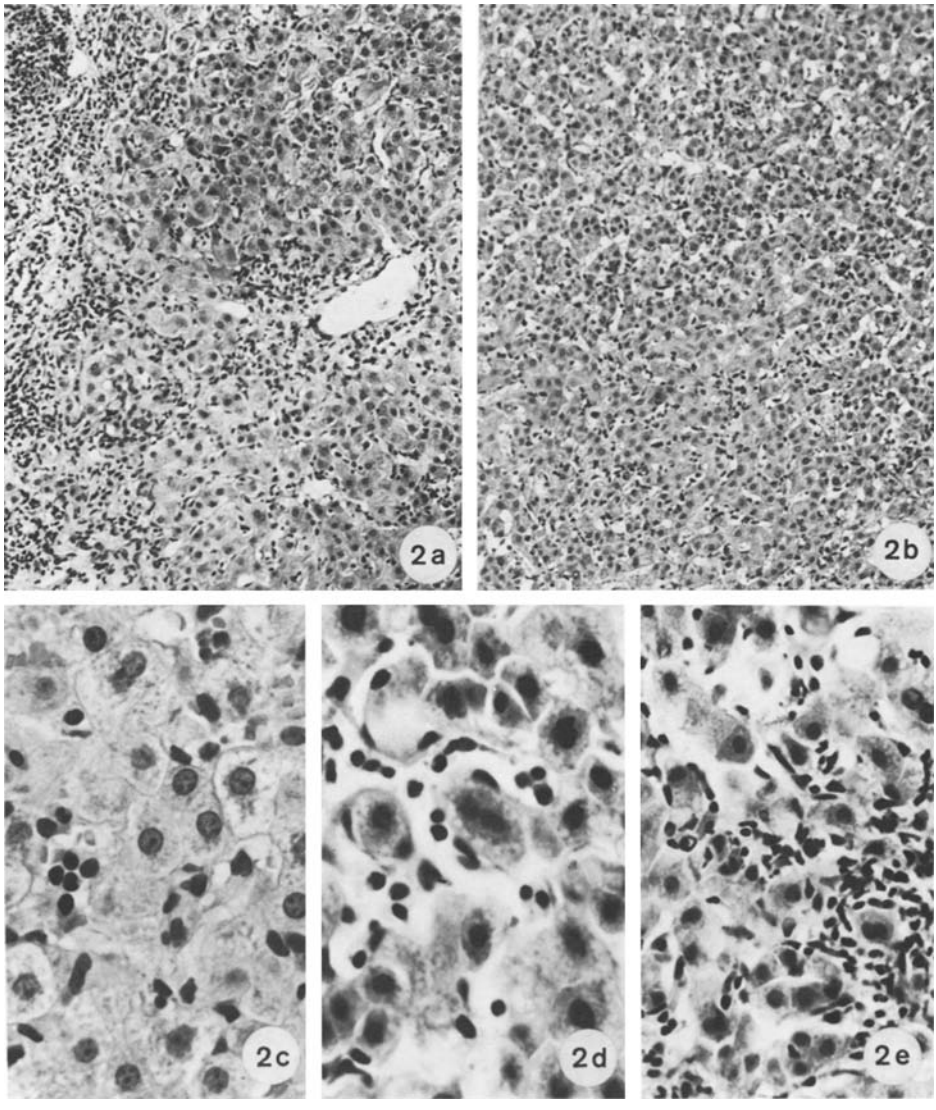


Fig. 2. Hepatitis nAnB (Padova). **a** The association of portal hepatitis with piecemeal necrosis and the lobular alterations of acute hepatitis is designated as acute hepatitis with signs of possible transition to chronicity (AHTC). (HE × 120) **b** A dense diffuse lymphohistiocytic intrasinusoidal infiltration is present throughout the lobule (scale 5). (HE × 120). **c** Mild intrasinusoidal lymphohistiocytic infiltrate; such degree was graded on scale 2. (HE × 300) **d** Moderate infiltrate, scale 4. (HE × 300) **e** Severe infiltrate, scale 5. (HE × 275)

posteriori selection tends to capitalize on chance. Statistically significant differences between cases from Leuven and Padova could not be documented for hepatitis A or B (Fig. 3, arrow 7). The total hepatitis A group from Leuven (L) and Padova (P) tended to show a lower degree of ballooning in comparison to the total hepatitis B group (L + P) ($p < 0.01$, Mann Whitney

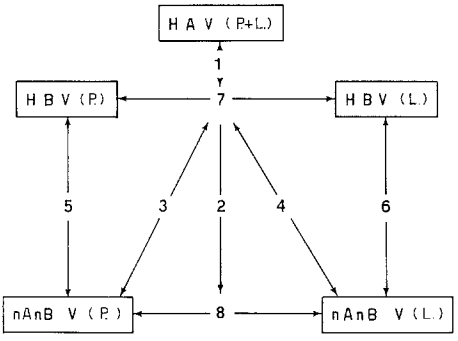


Fig. 3. Schematic representation of the various comparisons made in the text. The total number of hepatitis B cases (Leuven and Padova) (Nr. *7*) is compared with the total number of hepatitis A (Nr. *1*) and hepatitis nAnB cases (Nr. *2*), with the nAnB cases from Padova (Nr. *3*) and Leuven (Nr. *4*). Nr. *5* compares the hepatitis B and nAnB from Padova, whereas Nr. *6* compares the same etiologic groups from Leuven. Further comparisons were made between hepatitis B from Leuven and Padova (Nr. *7*) and between hepatitis nAnB from both centers (Nr. *8*)

test; Fig. 3, arrow 1). When comparing the total hepatitis B group (L+P) to all the hepatitis nAnB cases (L+P) (Fig. 3, arrow 2) the following differences were found (Chi-square test):

– The occurrence of lymphocytes ($p=0.05$), plasma cells ($p<0.05$), histiocytes ($p<0.05$) and neutrophilic polymorphonuclears ($p<0.02$) was higher in the HBV group, but the total portal inflammatory infiltrate as a whole was not statistically different.

– Bile duct proliferation was more prominent in the hepatitis B cases ($p<0.02$).

– Diffuse lymphocytic sinusoidal infiltration was more abundant in the nAnB cases ($p<0.001$).

When the whole hepatitis B group (L+P) was compared to the nAnB cases of Padova (all being transfusion related) (Fig. 3, arrow 3), the same parameters were found to be different in a statistically significant way (Chi-square test). Besides the more prominent diffuse lymphocytic intrasinusoidal infiltrations, the intralobular histiocytes were also more frequent in the Padova nAnB biopsies ($p<0.02$). In addition, the occurrence of eosinophilic polymorphonuclears was found to be higher in the portal infiltrate of biopsies taken in hepatitis B cases ($p<0.05$), as was the periportal fibrosis ($p<0.01$).

The comparison of the whole HBV group (L+P) with the nAnB cases of Leuven (Fig. 3, arrow 4) showed only a statistically significant difference in the degree of histiocytic intralobular infiltration which was now more abundant in the HBV-biopsies ($p<0.05$, Chi-square test).

We could not identify significant differences between the hepatitis B and hepatitis nAnB biopsies from Leuven (Fig. 3, arrow 6). However, clear differences were seen between the hepatitis B and hepatitis nAnB biopsies from Padova (Fig. 3 arrow 5). Portal hepatitis was more severe in the hepatitis B group (P) ($p<0.01$, Chi-square test). The occurrence of portal lymphocytes ($p=0.002$), plasma cells ($p=0.129$), histiocytes ($p=0.0011$), neutrophilic ($p=0.016$) and eosinophilic ($p=0.035$) polymorphonuclears was more prominent in the hepatitis B group (P) (Fisher test). Zonal necrosis was more frequently seen in the hepatitis B cases (P) ($p=0.0007$, Fisher test).

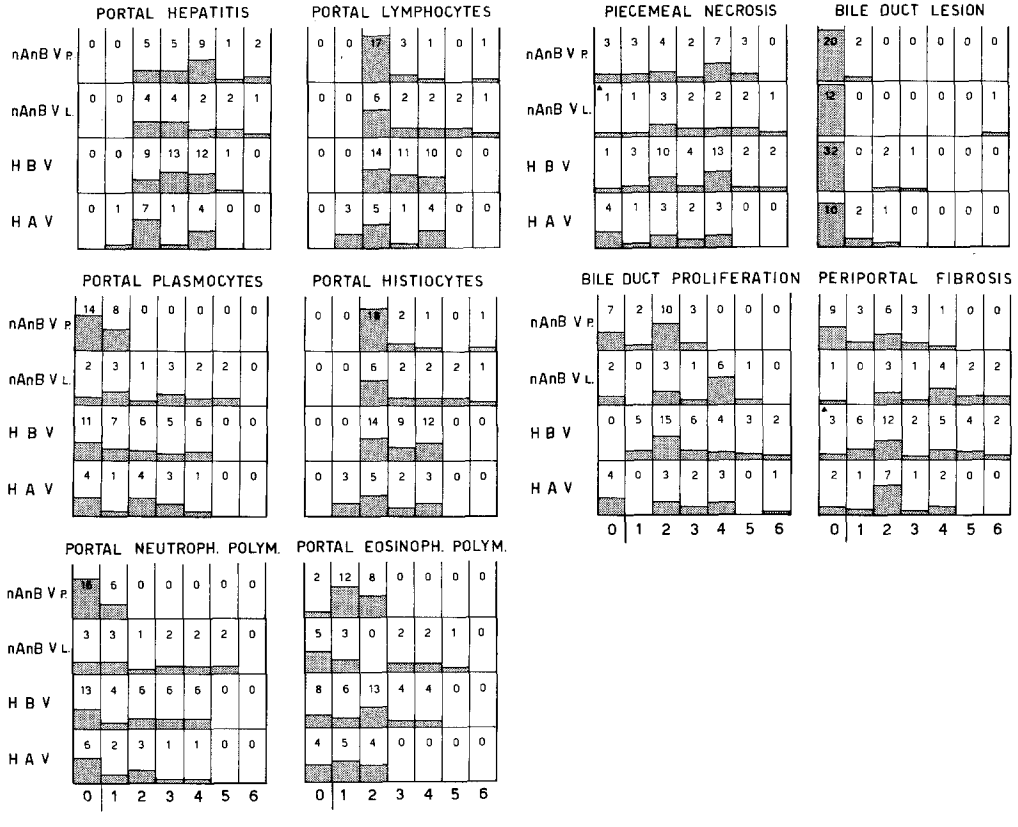
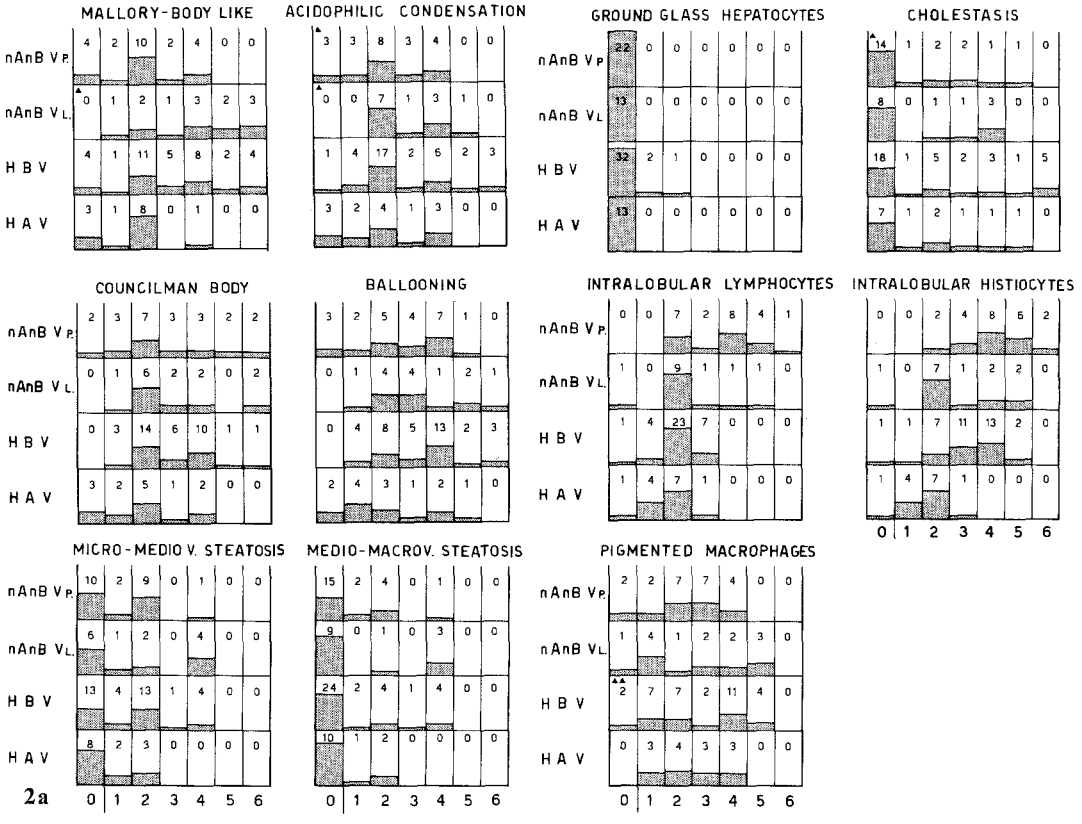


Table 2a

In nAnB viral hepatitis (P) the lymphocytic intralobular infiltration was more marked ($p = 0.0039$, Fisher test).

Cluster analysis of all the cases (hepatitis A, B, nAnB, from P+L) revealed a huge cluster in which the various aetiological groups mixed, except for the Padova nAnB cases. When only the nAnB cases were allowed, one homogeneous cluster appeared consisting of all Padova cases and four of the Leuven biopsies. The features of the remaining biopsies, all from Leuven, were more scattered than the Padova group. The differences, which were statistically significant (Chi-square test) between the two clusters, were characterized as follows: on the one hand a more conspicuous intrasinusoidal histiocytic ($p < 0.01$) and lymphocytic ($p < 0.05$) infiltration in the Padova-cluster, on the other a more heterogeneous portal inflammatory infiltrate comprising more plasma cells ($p < 0.001$), neutrophilic ($p < 0.001$) and eosinophilic polymorphonuclears ($p < 0.001$), Mallory body like cytoplasmic alterations ($p < 0.02$), bile duct proliferation ($p < 0.001$) and periportal fibrosis ($p < 0.001$) were more conspicuous in the Leuven biopsies.



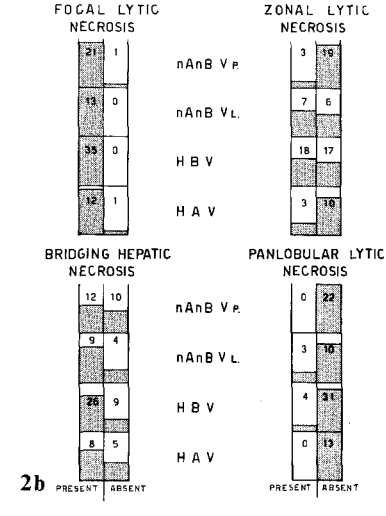


Table 2a, b. Summary of evaluation of histological parameters (except lytic necrosis) for the different etiological groups: hepatitis A virus (HAV), hepatitis B virus (HBV) and non-A, non-B virus (nAnBV). The latter is subdivided in two groups according to origin: Leuven (L) and Padova (P). The semi-quantitative scales (0–6) are given in the abscissa for each etiological group. The number of biopsies corresponding to a particular scale is indicated. The shaded blocks represent the same data calculated as percentage of the total number of biopsies per etiological group. ▲ = The number of triangles represents the number of biopsies in which this histological parameter could not be evaluated. (**b**) Presence or absence of focal, zonal, bridging hepatic and panlobular lytic necrosis in the different etiological subgroups. The number of biopsies and the calculated percentages are represented as in (**a**)

Discussion

Several authors have tried to define the histological characteristics of acute hepatitis A, B or nAnB both in experimentally infected marmosets or chimpanzees (Barker et al. 1975; Bianchi et al. 1982; Deinhardt et al. 1967; Dienstag et al. 1975; Holmes et al. 1969; Krawczynski et al. 1981; Popper et al. 1980) and in humans (Bamber et al. 1981; Kryger et al. 1980; Omata et al. 1981; Schmid et al. 1981, 1982; Teixeira et al. 1982). Others have looked for the differences in the histological alterations comparing acute hepatitis A, B and nAnB (Kryger et al. 1982; Phillips and Poucell 1981; Popper et al. 1980; Scheuer et al. 1980, 1981). Dienes et al. (1982) studied histological alterations in acute and chronic hepatitis nAnB compared with hepatitis A and B. Abe et al. (1982) described the light microscopic findings of liver biopsies from patients with hepatitis type A compared with type B. We have attempted to analyse the histological features in acute hepatitis occurring in Padova and Leuven. Cases of hepatitis A and B did not differ in Italy from cases in Belgium. Far less liver cell pleomorphism was found in hepatitis A than in B. Clear differences were found when acute hepatitis nAnB observed in Padova was compared with nAnB cases from Leuven or with hepatitis B (L+P). The portal inflammatory infiltrate was more heterogeneous in the hepatitis B group (L+P) and diffuse intralobular lymphocytic infiltration was more pronounced in the nAnB (L+P) biopsies. Ductular proliferation was more marked in the hepatitis B group (L+P), but this may be related to a later phase of acute hepatitis in this group, since 6 biopsies were taken between six and 12 months of clinical evolution. Comparison of the hepatitis B (L+P) group with the hepatitis nAnB group of Padova revealed in addition a more dense histiocytic intralobular infiltration in hepatitis nAnB (P) and a somewhat greater extent of periportal fibrosis in the total hepatitis B group. These differences were not observed when comparing the hepatitis B group with the nAnB hepatitis biopsies from Leuven.

Cluster analysis of the total nAnB group demonstrated a homogeneous cluster comprising the Padova cases, which were all transfusion-related, and 4 Leuven biopsies. One of this Leuven patients had a clinical history of blood-transfusions. The remaining cases, all nAnB biopsies from Leuven were not so homogeneous. The statistically significant differences between the nAnB cases of Padova and Leuven were: the presence of a higher intrasinusoidal histio-lymphocytic infiltration in the Padova nAnB biopsies, and a higher frequency of Mallory body like cytoplasmic eosinophilic condensations, a more heterogeneous portal tract inflammatory infiltrate and a more marked bile duct proliferation and periportal fibrosis in the Leuven nAnB biopsies. In this respect, the presence of a marked diffuse intrasinusoidal lymphocytic infiltration distinguishes the nAnB cases of Padova from all other biopsies analysed.

Various factors may be considered to explain the different histological patterns in the nAnB biopsies from Leuven and Padova such as: the virus, host reaction, modes of infection, timing of the biopsy and use of different

fixatives. The virus(es) which cause(s) the nAnB hepatitis in Padova may differ from the one(s) in Leuven. This may explain the overall histological differences observed between the two groups. The possible existence of two distinct agents in nAnB hepatitis has been already proposed from cross-challenge studies in chimpanzees (Bradley et al. 1980; Hollinger et al. 1980; Tsiquaye and Zuckerman 1979; Yoshizawa et al. 1981), from results of clinical studies (Bortolotti et al. 1982; Craske et al. 1978; Khuroo 1980; Mosley et al. 1977), from differences in incubation periods (Shimizu et al. 1979) and from different ultrastructural changes (Shimizu et al. 1979). These different documentations of the possible existence of more than one agent causing nAnB hepatitis are summarised in a recent paper by Holland and Alter (1981). The host reaction to a single nAnB virus may differ from one population to another. The reaction in Padova resembles the infectious mononucleosis type (Bamber et al. 1981; Gowing 1975; Phillips and Poucell 1981; Popper et al.1980; Scheuer et al. 1980), whereas the reaction pattern in the Leuven patients is not clearly different from that seen in acute hepatitis B in Leuven and Padova. The Padova nAnB hepatitis group comprises multiply transfused patients (Realdi et al. 1982), whereas most patients from Leuven had endemic sporadic hepatitis. These different ways of infection may represent infection with different viruses or with a different infectivity dose which is more massive in post-transfusion hepatitis cases (Bortolotti et al. 1982) and gives rise to different patterns of host reaction. Differences in the timing of the biopsy with regard to the acute clinical onset of the disease may explain some of the observed histological variations. The Padova patients were usually biopsied early (1–6 months) in the course of their disease, as part of a prospective study of post-transfusion hepatitis. In contrast, the presence of a more pronounced bile duct proliferation and periportal fibrosis in the Leuven biopsies may be explained by the fact that some of the latter biopsies were taken after more than 6 months of clinical evolution (Table 1). The use of different fixatives (neutral formalin in Padova and Bouin in Leuven) may also be responsable for some differences such as a better detection of Mallory body like eosinophilic cytoplasmic condensation in the Leuven biopsies. In spite of these various possibilities, the major differences between acute nAnB hepatitis in Padova ("mononucleosis" type) and in Leuven ("acidophilic body" type) are most probably related to differences in viral strain and different modes of transmission of disease.

Previous studies have emphasized some histological features as bearing some specificity for acute nAnB hepatitis. These include bile duct lesions of the Poulsen-Christoffersen type (Poulsen and Christoffersen 1972; Schmid et al. 1982), presence of giant cells (Schmid et al. 1982), diffuse lymphocytic intralobular infiltration (Bamber et al. 1981; Phillips and Poucell 1981; Popper et al. 1980; Scheuer et al. 1980), steatosis (Bamber et al. 1981) and fragmented "Councilman-like bodies" (De Wolf-Peeters et al. 1981). Bile duct lesions of the Poulsen-Christoffersen type were present in 9 of 83 biopsies in the present study; but the lesion was equally devided over hepatitis A (3/13), B (3/35) and nAnB (3/35). Giant cells were not

present in any of the biopsies analysed. Diffuse lymphocytic intralobular infiltration was observed in almost all cases of acute nAnB hepatitis in Padova and Leuven, but the degree of this infiltration was more severe in most of the Padova biopsies. Steatosis was not present to a higher extent in the nAnB biopsies included in this study. A different incidence in acidophilic bodies and Mallory body-like eosinophilic condensation of the cytoplasm was not observed in the present study when comparing the hepatitis B with the hepatitis nAnB group. The results of this study imply a close similarity of the histological changes in acute hepatitis B and acute nAnB hepatitis in Leuven. The similarity between these two groups might be explained by assuming a double viral infection (B+nAnB) in the hepatitis B group, creating a picture which is the addition of the characteristics of hepatitis B and nAnB (Dienes et al. 1981). This possibility can neither be proven nor excluded.

In conclusion, two different histological patterns were recognized in acute hepatits nAnB in this study:

1) One form mainly characterized by a high degree of intrasinusoidal lymphocytic infiltration (Padova cases), as was also observed elsewhere (Omata et al. 1981; Popper et al. 1980) and possibly related to transfusion-induced viral infection.

2) The other form showing a picture of acute hepatitis with numerous acidophilic bodies and Mallory body-like cytoplasmic alterations (Leuven type) not distinguishable from some cases of acute hepatitis B (L+P) and more easily detectable when Bouin fixative was used.

Furthermore, giant cells were not detected and the Poulsen-Christoffersen bile duct lesion did not appear to be specific in the present study. These observations suggest a double viral aetiology for nAnB hepatitis: one virus mainly transmitted by blood transfusions (Padova patients), a second virus more often involved in sporadic cases, and presumed to be closely related in morphology to the hepatitis B virus (Leuven patients).

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