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Brain Amino Acid Abnormalities in Liver Disease—A Postmortem Study

ABSTRACT: In a postmortem exploratory study, we examined whether specific amino acid abnormalities associated with liver diseases *in vivo* may also be detected in human brain samples obtained at clinical autopsies. The branched-chain amino acids (BCAA: valine, leucine, isoleucine) were decreased in the group of patients with liver diseases compared with the control group, whereas the aromatic amino acids (AAA: phenylalanine, tyrosine) were increased. However, the ranges overlapped significantly and were not statistically different. The molar ratio BCAA/AAA was determined to be 1.92 in the collection of patients with liver diseases compared with 2.27 in the control group. In patients with liver disease, ornithine concentrations in the brain appeared significantly decreased whereas glutamine was significantly increased. No significant difference was found in the brain concentrations of proline. Amino acid analysis may contribute to the understanding of pathophysiological mechanisms of liver disease, which are discussed, and may supplement the postmortem diagnosis.

KEYWORDS: forensic science, liver disease, amino acids, hepatic encephalopathy, edema

The liver is the major organ in the control of the protein and amino acid metabolism and various metabolic abnormalities in these substances occur during liver disease. The present postmortem study was intended to examine whether specific amino acid abnormalities associated with liver diseases *in vivo* may also be detected in human brain samples obtained at clinical autopsies, thus providing an additional tool of postmortem diagnosis.

Our interest was focused on three special hypotheses concerning amino acid metabolism and liver disease:

1. In chronic liver disease, the aromatic amino acids (AAA) tyrosine (TYR) and phenylalanine (PHE), which are mainly catabolized in the liver, are increased in plasma, whereas the branched-chain amino acids (BCAA) valine (VAL), leucine (LEU), and isoleucine (ILE), which are basically broken down in the muscles and fatty tissues, are moderately decreased (1). The plasma imbalance of these compounds could influence their reciprocally competing passage across the blood-brain barrier, thus leading to brain accumulation of the AAA. The AAA are precursors of monoamine neurotransmitters and changes in the levels of these compounds are of interest suggesting that disturbances in brain observed during hepatic encephalopathy may be partly due to alterations of these neurotransmitters (Fig. 1).
2. Brain edema remains a major cause of mortality in acute liver failure and it has been suggested that ammonia is of interest in this phenomenon (2). Ornithine (ORN) is a substrate for the urea cycle as well as for ornithine-α-ketoglutarate amino transferase and glutamate synthesis (Fig. 2). Glutamate (GLU) formed from ORN is available for glutamine (GLN) synthesis in skeletal muscle, which is considered as the major pathway for ammonia detoxification. Thus GLN and ORN levels were examined for evidence indicating the activation of this metabolic pathway.
3. In liver cirrhosis, when active collagen synthesis takes place, levels of free proline (PRO) and hydroxyproline are increased (3).

Materials and Methods

Brain free amino acid levels were measured in 199 cases from clinical autopsies. This collective included 18 patients with liver diseases, the demographic data are summarized in Table 1. According to the clinical records, these patients had not yet developed clinical signs of hepatic encephalopathy. Thirty patients with cardiovascular causes of death without a history of liver or renal disorders, malignom or diabetes were used as controls. Tissue samples from the precentral gyrus of the frontal lobe, area 4 according to Brodmann, were obtained at clinical autopsies and were stored immediately at −20°C until analysis. The complete brain had to be preserved for a neuropathological examination. Therefore the specimen collected for amino acid analysis had to be easily accessible from the surface of the brain, had to be easily identifiable in spite of the variability of the cortical architecture, and had to be of minor importance for the essential neuropathological examination.

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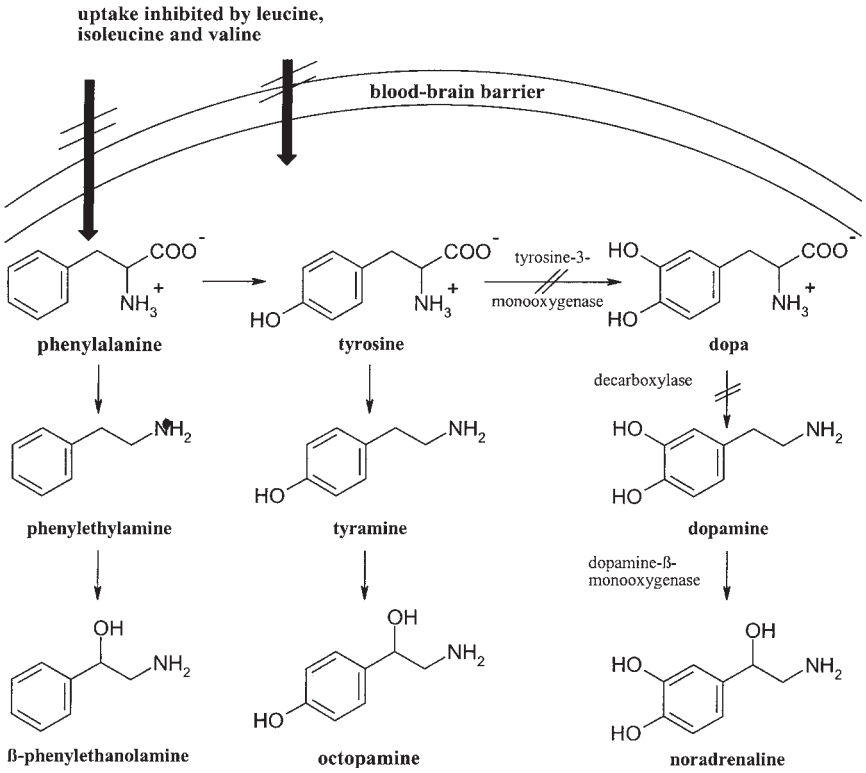

FIG. 1—*Biosynthesis of "false neurotransmitters" during hepatic encephalopathy. The AAA (TYR, PHE) are increased in plasma, whereas the BCAA (VAL, LEU, ILE) are moderately decreased. The plasma imbalance leads to brain accumulation of the AAA. Furthermore, the tyrosine-3-monooxygenase is inhibited by PHE, which leads to a reduced formation of dopamine and noraderenaline. The AAA are metabolized to the "false neurotransmitters" phenylethanolamine and octopamine. Modified according to (1).*

Sample Preparation

Brain tissue (100–200 mg wet weight) was homogenized in a solution of sulfosalicylic acid. The homogenate was then centrifuged at 2000 g for 10 min and the supernatant was adjusted at pH 2.2 by addition of a sodium citrate buffer (0.2 M) containing D,L-β-aminoisobutyric acid as internal standard. An aliquot was subjected to amino acid analysis.

Amino Acid Analysis

Analysis was determined using an automated Biotronic LC 6001 amino acid analyzer (Frankfurt a.M., Germany) with a BTC 2710 type ion exchange column, ninhydrine detection and lithium citrate buffers as mobile phases (4). Amino acid elution was accomplished with a modified six step program using five buffers and three temperatures. Step 1:9 min buffer A at 37°C; step 2: 35 min buffer B at 37°C; step 3: 28 min buffer C at 54°C; step 4: 8 min buffer D at 54°C; step 5: 50 min buffer E at 54°C; step 6: 35 min buffer E at 65°C. Li^+ and citrate concentrations and pH were set in the buffer A 0.15 and 0.10 M and 2.70, B: 0.15 and 0.10 M and 3.65, C: 0.15 and 0.10 M and 4.0, D: 0.55 and 0.08 M and 4.00, E: 1.40 and 0.08 M and 3.30, respectively. The overall reproducibility of results was consistent within ±5%.

Statistics

The results of the amino acids and AAA/BCAA were evaluated using the U-test according to Mann, Willcox and Whitney. A p value less than 0.05 was considered to be significantly different.

Results

In the analysis of 199 brain samples, we measured amino acid concentrations in the ranges described by other groups (Table 2) (5,6). We found no correlations between amino acid levels and sex or age (regression analysis).

The influence of the postmortem interval between time of death and autopsy showed an increase of free amino acid levels in the brain samples for a period greater than three days. For this reason for further purposes, we used only cases with a postmortem interval up to three days.

Branched-Chain Amino Acids—The BCAA were decreased in the group of patients with liver diseases compared with the control group (Fig. 3). However, the decrease in the concentrations was not statistically significant.

Aromatic Amino Acids—The AAA were increased in the group of patients with liver diseases compared with the control group (Fig. 4). However, the increase in the concentrations was not statistically significant.

Molar Ratio BCAA/AAA—The molar ratio (VAL + LEU + ILE)/(TYR + PHE) was determined to be 1.92 in the collective of patients with liver diseases compared with 2.27 in the controls. However, this was not considered to be significantly different.

Ornithine and Glutamine—In Fig. 5, the concentrations of ORN and GLN are described measured in the group of patients with liver diseases compared with those of controls. In patients with liver dis-

eases, ORN appeared significantly decreased whereas GLN was significantly increased.

Proline—No significant difference was found in the brain concentrations of PRO between patients with liver diseases and controls.

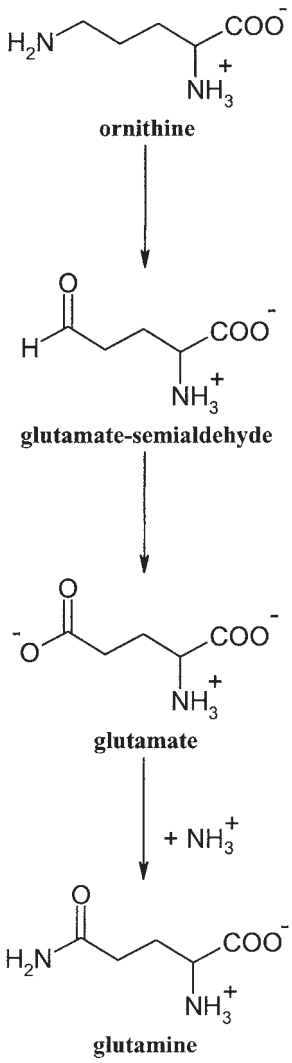

FIG. 2—*ORN is a substrate for both the urea cycle and for ornithine-α-ketoglutarate amino transferase. Then glutamate semialdehyde dehydrogenase transforms glutamate semialdehyde into GLU. This GLU formed from ORN is thus available for several reactions including GLN synthesis in skeletal muscle via glutamine synthase.*

Discussion

In the present study, the collective of patients with liver diseases and the control group were characterized by the clinical history, the findings of the autopsy, and the results of the microscopic examination. As for the liver disease group, one has to take into consideration that this collective includes diseases of the liver of different etiology. This diversity may contribute to the observed variability in the brain amino acid concentrations, but on the other hand, may be of minor relevance as the alterations of amino acid metabolism are mainly related to the loss of liver tissue (independent on the etiology). Furthermore, amino acid analysis is not intended to replace the postmortem morphological examination but to supplement the morphological findings with correlating biochemical results indicating the associated functional impairment.

As far as the control group is concerned, one has to take into account that the impairment of liver function may lead to alterations in the concentrations of plasma amino acids in humans with liver disease. For this reason, the most important criterion to select a control group is the absence of medical conditions that might exert any influence on the concentrations of amino acids in the brain. These conditions in particular, include diseases of the liver, the kidneys, and metabolic disorders while associations of cardiac diseases and amino acid metabolism are not known. As a consequence, it appeared reasonable to make up the control group out of fatalities due to cardiac diseases without additional pathological condition of liver and kidney and without metabolic disorder.

In chronic liver disease, the AAA are increased in plasma two- to four-fold, whereas the BCAA are moderately decreased (7). In 1971, Fischer and Baldessarini hypothesized that many of the manifestations of hepatic insufficiency could be explained by replacement of the true neurotransmitters dopamine and noradrenaline in the nervous system by false neurotransmitters such as octopamine and phenylethanolamine (8). An important factor in the control of neurotransmitter synthesis, especially in the adrenergic and serotonergic systems, is the brain concentration of the precursor amino acids, especially tyrosine, phenylalanine, and tryptophan. The free brain concentrations of these amino acids may be dependent upon their plasma concentrations, although competition for active entry across the blood brain barrier does occur, especially for the three BCAA (9–11). It was suggested that diminished noradrenaline synthesis might result from disturbances in the normal passage of neutral amino acids across the blood brain barrier (12). Furthermore, it was demonstrated that brain serotonin concentrations might be related to the ratio between tryptophan, its direct precursor, and five other amino acids competing with it for entry across the blood brain barrier. Therefore, plasma amino acid concentrations could be of

TABLE 1—*Demographic data of the liver disease group and the control collective.*

Collectives	Age (y)	Sex (m/w)	Postmortem Delay (d)	Cause of Death Respectively Liver Pathology
controls ($n = 30$) criteria of exclusion: • chronic diseases of the liver (hepatic failure) • chronic renal disease (renal failure) • metabolic disorders • neoplasia	44–91 mean 71	17 m/13 w	up to 72 h	cardiovascular disorder: • 8 × coronary atherosclerosis • 3 × myocardial infarction • 3 × aortic stenosis • 4 × aortic aneurysm • 7 × pulmonary thromboembolism • 5 × varia
patients with liver disease ($n = 18$)	35–85 mean 64	10 m/8 w	up to 72 h	• 12 × cirrhosis (5 × alcoholic, 7 × aetiology unknown) • 6 × tumors (carcinomas) with resulting liver disease

TABLE 2—*Brain amino acid concentrations determined in samples from 199 clinical autopsies (104 men, 95 women; mean age 66.5 y; prevailing causes of death: pneumonia (20.1%), myocardial infarction (14.6%), pulmonary thromboembolism (10.1%) gastrointestinal hemorrhage (6%), rupture of an aneurysm of the aorta (3.5%), malignant neoplasms in particular of the gastrointestinal tract (12.6%).*

Amino Acid	Mean (μmol/g)	±S.D.	Amino Acid	Mean (μmol/g)	±S.D.
Taurine (TAU)	3.028	1.532	Cysteine (CYS)	0.294	0.254
Phenylethanolamine (PEA)	0.751	0.516	Methionine (MET)	0.586	0.469
Urea (UREA)	1.957	1.493	Cystathion (CYSTA)	1.488	1.142
Aspartate (ASP)	5.391	2.689	Isoleucine (ILE)	0.921	0.567
Threonine (TRE)	1.701	0.932	Leucine (LEU)	1.873	0.986
Serine (SER)	3.270	1.946	Tyrosine (TYR)	1.046	0.497
Glutamate (GLU)	13.308	5.780	Phenylalanine (PHE)	1.041	0.592
Glutamine (GLN)	5.223	2.967	Homocysteine (HOMOC)	0.141	0.072
Proline (PRO)	2.996	2.102	γ-Aminobutyric acid (GABA)	1.799	0.958
Glycine (GLY)	4.195	2.228	Ornithine (ORN)	0.257	0.271
Alanine (ALA)	5.134	2.695	Lysine (LYS)	1.599	0.954
Citrulline (CIT)	0.321	0.289	Histidine (HIS)	0.544	0.316
α-Aminobutryic acid (ABU)	0.133	0.127	Tryptophan (TRY)	0.115	0.055
Valine (VAL)	1.996	1.132	Arginine (ARG)	1.441	0.748

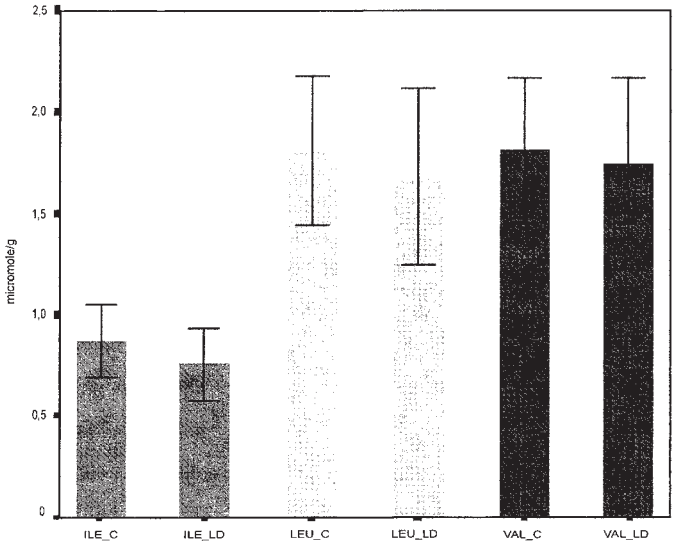

FIG. 3—*Concentrations of BCAA in the group of patients with liver diseases compared to the control group (ILE_C/LD, LEU_C/LD, VAL_C/LD = isoleucine, leucine and valine concentrations of the control collective (C) and liver disease group (LD)).*

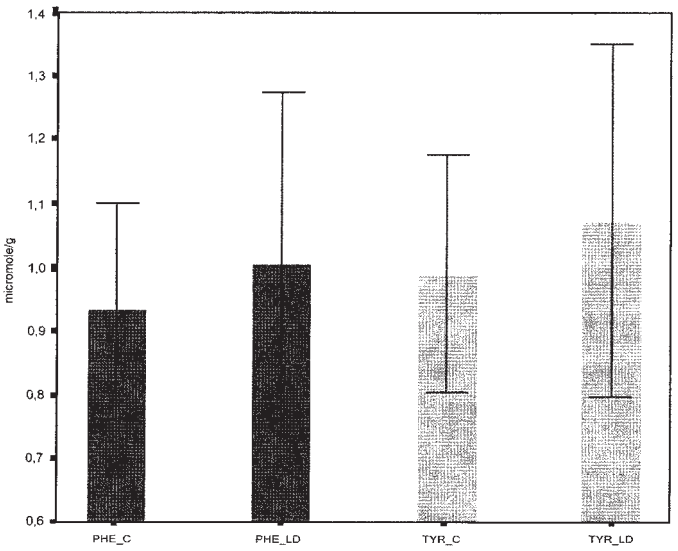

FIG. 4—*Concentrations of AAA in the group of patients with liver diseases compared to the control group (PHE_C/LD, TYR_C/LD = phenylalanine and tyrosine concentrations of the control collective (C) and liver disease group (LD)).*

great importance in hepatic encephalopathy. Specific plasma amino acid patterns have been demonstrated in patients with chronic hepatic insufficiency and encephalopathy with increased concentrations of the AAA and decreased concentrations of the BCAA. Fischer et al. (13) found that the molar ratio between concentrations of the BCAA and the AAA tended to be constant. Under normal conditions, the mean ratio was 3.0 to 3.5 whereas in patients with hepatic encephalopathy, the mean ratio was significantly reduced (7,14–19). According to Soeters and Fischer (20) hepatic encephalopathy might be caused by changes in the plasma concentrations of the five amino acids considered in this ratio.

Increases in both AAA and BCAA have been found in the frontal cortex of patients who died with fulminant hepatic failure (21). Otherwise in brain tissues of cirrhotic patients, no changes in BCAA concentrations but an increase in AAA levels were observed compared with controls (22). In our study, we found increased levels of AAA accompanied by a decrease in the BCAA. The molar ratio BCAA/AAA was determined to be 1.92 in the collective of patients with liver diseases compared with 2.27 in the controls. However, all of these findings were not considered to be significantly different. Whether or not increases in AAA are associated with changes in monoamine neurotransmitters in the brain of patients with liver disease (hepatic encephalopathy) awaits further studies. It should be mentioned that in the present study, the concentrations of BCAA and AAA were determined only in brain tissue samples and not in plasma samples. However, a complementary examination of the plasma levels might provide a tool to study the pathophysiological mechanisms of brain amino acid changes in hepatic failure in the future.

Brain edema sufficient to cause intracranial hypertension and brain herniation remains a major cause of mortality in acute liver failure. It has been suggested that ammonia plays a predominant role in this phenomenon (23–25). Furthermore, it has been demonstrated that administration of L-ornithine-L-aspartate (OA) leads to a significant lowering of plasma and CSF ammonia in experimental and human chronic liver failure (26–28). The ammo-

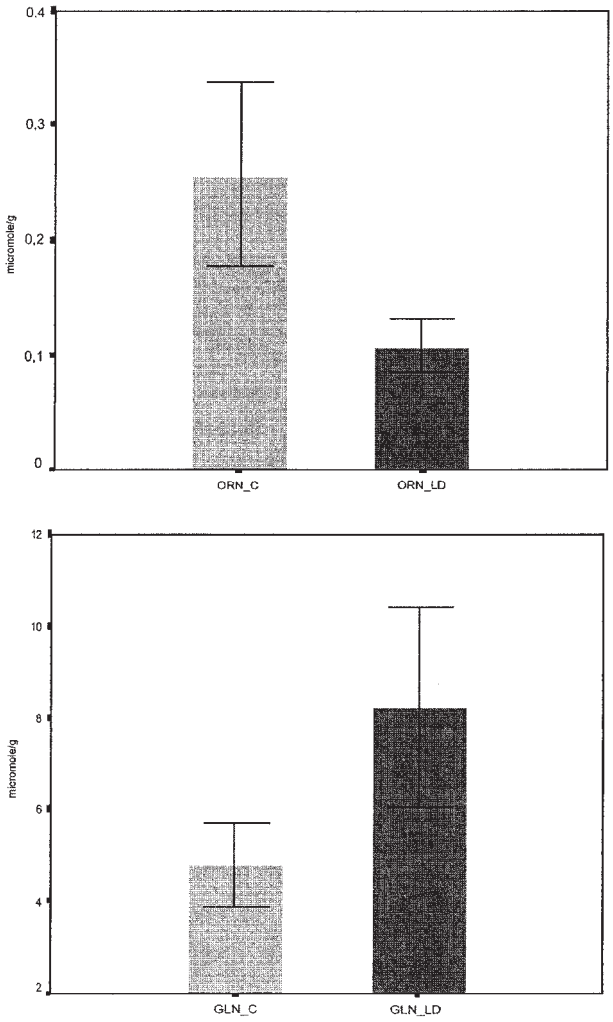

FIG. 5—*Concentrations of ORN and GLN in the group of patients with liver diseases compared with those of control group (ORN_C/LD, GLN_C/LD = ornithine and glutamine concentrations of the control collective (C) and liver disease group (LD)).*

nia-lowering effect of OA was accompanied by a reduction in brain water content and a delay in the time of onset of severe encephalopathy. Furthermore, a two-fold increase of plasma GLN was observed (29). These findings suggested that OS stimulates glutamine synthesis in the periphery (skeletal muscle). It has been reported that in severe liver failure, muscle ammonia uptake is enhanced and that the subsequent GLN synthesis is the major pathway for ammonia detoxification (30). In our study in patients with liver diseases, ORN appeared significantly decreased whereas GLN was significantly increased. An increase of GLN brain concentrations in patients with liver disease was also described by others (31). In our opinion, these findings are indicative for an induced ammonia intoxication in patients with liver disease. The detoxification pathway via ORN to GLN is markedly enhanced.

The amount of free PRO in the liver may play an important role in regulation of collagen biosynthesis in fibrogenesis such as liver cirrhosis (32–34). Under pathological conditions in which collagen metabolism is maintained constant in bone and skin, it is still possible to detect modifications in the plasma values of PRO and hydroxyproline in patients with liver disease. It was demonstrated that free PRO and hydroxyproline were significantly increased in alcoholic cirrhotics (3). In several patients with high plasma values of both amino acids who stopped drinking alcohol, the free amino acids decreased significantly. These findings suggested that ethanol by itself is an important factor not only in the pathogenesis of cell damage but also in fibrinogenesis (3,35,36). In our study, we did not find significant differences in the brain concentrations of PRO between patients with liver diseases and controls. However, such findings could only be connected with active states of fibrinogenesis. A further explanation for our results is given by the findings of Kruse (37) who studied the amino acid transport across the human blood-CSF barrier. For PRO (and hydroxyproline), a special control system was suggested because of their smaller biological variance in CSF than in blood. According to the authors, one possibility could be that these amino acids have a variation of metabolic turnover in blood which is faster than the equilibration between blood and CSF. A second possibility could be a local control mechanism in the brain.

In summary, the present postmortem study resulted in positive evidence for an increase of AAA in patients with liver disease that may be due to alterations in neurotransmitter concentrations with further effects for the course of the illness. However, to use the values obtained in the present study for practical medicolegal issues is hampered by the fact that data for the AAA and BCAA for the controls versus the patients are overlapping. As far as the nonsignificant findings are concerned, one has to consider that the patients examined had not yet the signs of hepatic encephalopathy. One might draw the conclusion that imbalances in the amino acid metabolism preceed the occurrence of clinical symptoms, but reach statistical significance at the time, when the clinical diagnosis may be established. Especially, we got evidence for an ammonia intoxication in patients with liver disease, which is of interest in the pathogenesis of brain edema in acute liver failure. Amino acid analysis may contribute to the understanding of pathophysiological mechanisms of liver disease and may supplement the postmortem diagnosis.

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