Paper IV

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Biochemical and Cytologic Analysis of Cystic Contents in Benign Non-Parasitic Symptomatic Hepatic Cysts Before and After Ethanol Sclerotherapy

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Purpose: To examine the fluid of liver cysts by cytologic and biochemical analysis before and after ethanol sclerotherapy in order to explore the etiology of cystic fluid reproduction after sclerotherapy.
Material and Methods: The contents of 11 cysts in 11 patients were examined on the day of sclerotherapy, and 2–8 (mean 4.5) days later, and analysed for cytologic and biochemical parameters.
Results: Cytologic signs of acute or subacute inflammatory reaction were absent before and present in all cysts after sclerotherapy. Biochemical parameters reflecting the acute inflammatory reaction (CRP, orosomucoid and haptoglobine), changes in capillary permeability (protein, albumin), and the cystic epithelial function (bilirubin, alkaline phosphatase) were significantly elevated after sclerotherapy.
Conclusion: The post-sclerotherapy fluid production is probably due to an inflammatory reaction. This may explain the success of performing sclerotherapy in one single session.

Key words: Alcohol, cyst; percutaneous drainage; liver, cysts; liver, interventional procedure

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Hepatic cysts are remnants of fetal biliary ducts and are lined by a single-layered cuboidal epithelium with functional characteristics of biliary epithelium (4). When symptomatic liver cysts are drained, they recur within weeks or months (12). However, when drainage is combined with sclerotherapy, a permanent and satisfactory volume reduction is achieved (1, 2, 5, 8–11, 14–16). Therefore percutaneous sclerotherapy has become the method of choice in the treatment of symptomatic hepatic cysts (1, 2, 5, 8–11, 14–16). Surgery, too, is efficient (3, 7, 13), but sclerotherapy has other benefits compared to surgery, especially concerning costs and risk. Ethanol (95–99%) has been the sclerosing agent most commonly used (1, 2, 5, 8–11, 14–16).

Some authors suggest that post-sclerotherapy fluid production is a sign of remnant vital cyst wall epithelium. As a consequence, they perform additional sclerotherapy sessions (1, 16). As many as 11 consecutive sclerotherapy procedures of one single cyst by means of a catheter left in place for 41 days was reported in one study (16). Other investigators consider the post-sclerotherapy fluid production to represent leakage from the cystic surface due to an ethanol-induced inflammatory reaction limited in time (2, 5, 8–11, 14, 15).

The aim of the present study was to examine the content of cystic fluid before and after sclerotherapy in order to evaluate the etiology of cystic fluid reproduction.

Material and Methods

Patients

During two periods, the first from September 1998 until November 1999 and the second from May

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2002 to September 2002, cystic contents from all patients referred for ethanol sclerotherapy were analyzed by cytologic and biochemical methods before and after sclerotherapy. Patients with polycystic liver disease and abdominal pain, but without large cysts suitable for sclerotherapy, were not included. Patients with hydatid cyst, neoplastic cysts and cyst-like ectasies of the intrahepatic biliary ducts (Carolis disease) were excluded. Informed consent was given by all patients.

Eleven cysts in 11 patients (9 women and 2 men), mean age 63.4 (45.2–85.8 years) were included. Six patients suffered from polycystic disease of the liver. Five patients had solitary or few liver cysts. Only one cyst in each patient was punctured. Two cysts, both from livers containing few cysts, were examined only by biochemical methods because too many erythrocytes made cytologic examination impossible. Thus 9 cysts were examined by cytologic and 11 by biochemical methods.

Procedural aspects

Sclerotherapy was performed using a catheter indroduced under ultrasonographic and fluoroscopy guidance (8, 9). The cystic fluid was completely evacuated. A sample was taken from this fluid for analysis. Immediately thereafter, 96% of ethanol was instilled. The volume of the applied 96% ethanol represented usually 10% of the cyst volume, but never more than 100 ml. Cysts with volumes below 1000 ml were exposed to ethanol for 10 min. Cysts with volumes >1000 ml were twice exposed to 100 ml ethanol for 10 min each session – 20 min in total. Thereafter all ethanol was evacuated. After irrigation with saline the catheter was removed. The second sample was collected from 2 to 8 (mean 4.4) days later by means of a needle with 0.7 mm outer diameter (Chiba biopsy needle with echogenic tip; Medical Device Technologies Inc., Gainesville, Fl., USA). The cytologic and biochemical analysis performed are listed in Table 1.

Cytologic examination

The fluid sample was divided into two portions, one fixated in 70% ethanol and stained according to Papanicolao, the other unfixated stained according to Diffquick. The cellular counting was done manually. Technologists trained for cytologic examination counted each cellular element. When 200 cellular elements were counted, the relative percentual distribution of neutrofile and eosinophile granulocytes, lymphocytes, and macrophages was calculated. The technologist's report was finally evaluated by a pathologist with experience in cytologic diagnostic work.

Biochemical examination

The cystic fluid was examined by nine different biochemical parameters both before and following sclerotherapy. The biochemical parameters used reflect the acute inflammatory reaction, changes in capillary permeability, the cystic epithelial function, and the degree of liver tissue damage (Table 1).

A consecutive analysis of the same parameters in blood as in cystic fluid before and after sclerotherapy was done only in the last five patients examined.

Statistical analysis

The differences between the observed data before and after sclerotherapy were examined using the Wilcoxon signed rank test for non-parametric statistics. All *P*-values are two-tailed.

Results

Cytologic examination

The results of the cytologic examination are listed in Table 2. Preceding sclerotherapy, a percentual calculation of cellular elements was impossible in eight cysts because the fluid was completely acellular in six and contained only a few lymphocytes in two (Table 2). Only in one cyst (cyst number three) was a percentual calculation of

Table 1. Parameters applied in the present evaluation of ethanol sclerotherapy of benign liver cysts

Cytologic examination
A semi-quantitative, manual method used for counting and characterization of inflammatory cells. The same method as used in bronchial lavage
Biochemical tests
Parameters reflecting the acute inflammatory reaction
CRP* (most specific parameter)
Orosomucoid
Haptoglobin (least specific parameter)
Parameters reflecting increased capillary permeability, the leakage of macromolecules
Protein
Albumin
Parameters indirectly reflecting cystic epithelial damage
Alkaline phosphatase
Bilirubin
Parameters of liver tissue (hepatocyte) damage
ALT** (most specific parameter)
AST*** (less specific parameter)

*CRP (C-reactive protein). An acute phase protein with elevated values in bacterial infections and tissue necrosis. **ALT (alanine-amino transferase). The most specific liver enzyme with regard to hepatocyte damage. ***AST (aspartate amino transpherase). Less liver specific enzyme because hemolysis may result in high values.

Table 2. Cytologic examination of cystic contents before and after ethanol sclerotherapy

Patient no.	Days between cytologic examinations	BEFORE Cellular elements in pct	AFTER Cellular elements in pct		
			Lymph	N.gr.	Ma.ph
I	2	Some few lymphocytes	0	100	0
II	2		0	100	0
III	2	100% lymphocytes	0	98	2
IV	2	Some few lymphocytes	2	98	0
V	2	0	11	80	9
VI	6	0	15	70	15
VII	7	0	30	68	2
VIII	7	0	25	71	4*
IX	8	0	25	60	15

Lymph. = Lymphocytes. N.gr. = Neutrophil granulocytes. Ma.ph. = Macrophages.
*4% eosinophil granulocytes.
The interval between the first and second samples was 2–8 days (mean 4.2 days).

cellular elements possible, yielding 100% lymphocytes (Table 2). The post-sclerotherapy cytologic examination demonstrated an increase in the number of cellular elements in all cysts, thus allowing the relative percentages of different kinds of leukocytes to be calculated (Table 2). Neutrophil granulocytes (98–100%) were present in 4 out of 5 cysts, with an interval of only 2 days between the first and the second sample. In one cyst with an interval of 2 days and in all 4 cysts with an interval of 6, 7, or 8 days between the pre- and post-sclerotherapy sample, the dominance of neutrophil granulocytes was partially replaced by some lymphocytes and macrophages. In one cyst the finding of 100% lymphocytes was replaced by 98% neutrophil granulocytes and 2% macrophages 2 days following sclerotherapy.

Biochemical analysis of cystic fluid

The results of the biochemical analysis of cystic fluid before and after sclerotherapy are given in Table 3. Parameters reflecting both acute inflammatory reaction (C-reactive protein (CRP), haptoglobin, and orosomucoid) and cyst epithelial function (bilirubin and alkaline phosphatase (ALP)) and macromolecular leakage (protein and albumin), as well as parameters of hepatocyte function (alanine-amino transferase (ALT) and aspartate-amino transferase (AST)) increased significantly ($P \leq 0.05$) following ethanol sclerotherapy (Table 3).

Biochemical analysis of blood

In the last five patients an analysis of the same biochemical parameters in blood was performed both before and following sclerotherapy (Table 4). The median CRP value in blood was 7 before sclerotherapy and 71 after. The median values of CRP in cystic fluid in the same patients were 9 (range 0–10) before sclerotherapy and 10 (range 5–24) following sclerotherapy. The second blood sample was taken 2–3 days, mean 2.2 days after sclerotherapy. The other parameters only demonstrated

Table 3. Biochemical analysis of cystic contents from 11 hepatic cysts in 11 patients before and after ethanol sclerotherapy

Laboratory test	Before sclerotherapy		After sclerotherapy		*P*-value
	Range	Mean	Range	Mean	
CRP (mg/l)	0–10	4	0–35	15	0.013
Orosomucoid (g/l)	0.01–0.88	0.32	0.45–1.08	0.710	0.005
Haptoglobin (g/l)	0.08–1.19	0.3091	0.24–1.19	0.650	0.005
Albumin (g/l)	1–33	13.2	21–29	26.2	0.016
Protein (g/l)	3–52	20.5	33–48	41.8	0.010
Bilirubin (μmol/l)	0–7	3.5	7–35	18.6	0.003
Alkaline phosphatase (U/l)	0–4	1.5	28–185	103.4	0.003
ALT (U/l)	0–27	4.5	11–84	27	0.004
AST (U/l)	2–122	19.6	22–165	62	0.021

The interval between the first and second samples was 2–8 days (mean 4.4 days).
The differences between the observed data before and after sclerotherapy were examined using the Wilcoxon signed rank test for non-parametric statistics. All *P*-values are two-tailed.

Table 4. Biochemical analysis of blood in five conscutive patients before and after ethanol liver cyst sclerotherapy

Laboratory test	Before sclerotherapy		After sclerotherapy		*P*-value
	Range	Median	Range	Median	
CRP (mg/l)	1–8	7	18–153	71	0.043
Orosomucoid (g/l)	0.61–1.08	0.79	1.02–1.54	1.08	0.043
Haptoglobin (g/l)	0.82–2.22	1.16	1.39–2.86	0.71	0.043
Albumin (g/l)	32–45	42	37–41	39	0.496
Protein (g/l)	59–76	73	68–74	69	0.498
Bilirubin (μmol/l)	7–18	12	14–27	16	0.102
Alkaline phosphatase (U/l)	180–455	241	159–546	271	0.686
ALT (U/l)	17–50	21	17–167	34	0.068
AST (U/l)	20–37	23	19–95	29	0.465

The interval between the pre- and post-sclerotherapy blood sample was 2–3 days (mean 2.2 days). The differences between the observed data before and after sclerotherapy were examined using the Wilcoxon signed rank test for non-parametric statistics. All *P*-values are two-tailed.

minor differences in the values before and following sclerotherapy.

Complications

No complications were seen.

Discussion

At cytologic examination of cystic contents before sclerotherapy, signs of acute or subacute inflammatory reaction were absent in all cysts. In one cyst a large number of lymphocytes indicated a chronic inflammatory response. Following sclerotherapy, cytologic signs of acute or subacute inflammation were present in all nine cysts.

Different biochemical parameters reflect different aspects of the pathophysiology of the ethanol sclerotherapy procedure. The statistically significant increase of CRP, orosomucoid, and haptoglobin is of particular importance since these reflect the inflammatory response. CRP is the most specific indicator of inflammation and therefore also the most important of these three parameters. Orosomucoid is less specific than CRP, and haptoglobin is an even less specific indicator of acute inflammation.

The increase in protein and albumin concentration in cystic contents indicates a macromolecular leakage due to increased gaps between the endothelial cells in capillaries and venules. This is an important sign of an inflammatory reaction. The increased leakage of water, minerals, and macromolecules is essential in reproducing cystic contents post-sclerotherapy. Therefore albumin and protein are probably of similar importance in the present investigation as the acute phase reactants CRP, haptoglobin, and orosomucoid. In a study by KALIA (6), exposure of the rat gastric mucosa to 60% ethanol resulted in mast-cell degranulation and histamine release, hyperemia, and microvascular damage with increased gaps between the endothelial cells, resulting in leakage of fluid and macromolecules. Probably similar effects occur in the epithelium of human liver cysts.

ALP and bilirubin are probably indirect indicators of damage to the epithelium of the cystic wall. When the single-layered epithelial lining is devitalized due to alcohol, bilirubin and ALP may leak from the adjacent liver tissue into the cystic cavity. The differences between pre- and post-sclerotherapy values of these parameters might support the theory of devitalization of cystic epithelium by single-session exposure to ethanol.

Among the biochemical parameters selected, ALT is the main indicator of liver cell function. The elevation of ALT indicates that the ethanol-induced damage has not only affected the cyst epithelium but also the adjacent liver tissue. This minor liver tissue damage is not of clinical importance. Moderate or severe liver tissue damage as a complication of liver cyst ethanol sclerotherapy has not been observed during our experience with liver cyst sclerotherapy procedures in 44 patients. Severe liver tissue damage as a complication of ethanol liver cyst sclerotherapy has not been reported in the literature (1, 2, 5, 8–11, 14–16).

The interval between the first and second puncture varied between 2 and 8 days. This did not create problems regarding interpretation of the results of the cytologic examination, because signs of acute or subacute inflammatory reaction were absent in all cysts before sclerotherapy and present in all following sclerotherapy. Also a post-sclerotherapy increase of statistical significance was observed in cystic fluid in all nine different biochemical parameters evaluated.

Only in the last five patients was an analysis of the same biochemical parameters done in both

blood and cystic fluid before and following sclerotherapy. The mean CRP value in blood was normal before sclerotherapy and significantly elevated afterwards. The mean values of CRP in cystic fluid in the same patients were 9 before and 10 following sclerotherapy. This is a sign of a significant tissue reaction in blood but not in cystic fluid. The explanation as to why CRP values in blood were higher than in cystic fluid might be that the cystic wall is vascularized, so allowing the CRP to get into the circulating blood while the cystic fluid is avascular, probably making CRP elevation difficult. The other biochemical parameters examined in blood only demonstrated minor differences in the values before and following sclerotherapy. In particular, there were only slight signs of liver tissue damage.

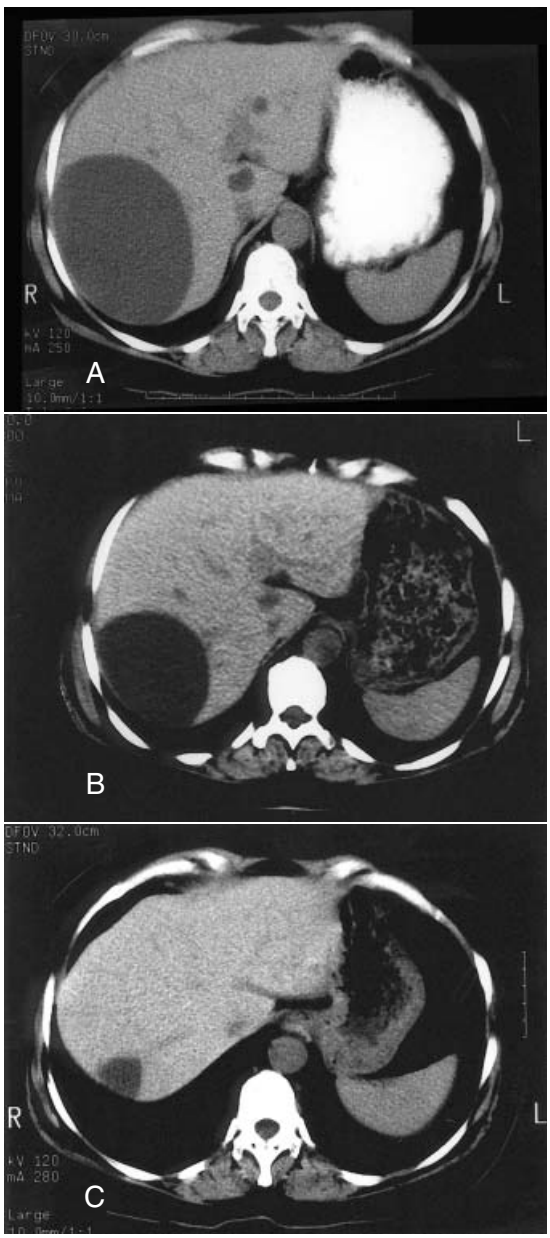

Fig. 1. A. In a 37-year-old female patient, a 400 ml symptomatic liver cyst located in the right lobe of the liver was treated by single-session ethanol sclerotherapy. B. Three months later the volume was 160 ml, representing a partial recollection of cystic contents (40% of the original volume).The present study investigated the mechanism and nature of this recollection of fluid. C. Without repeating the sclerotherapy procedure, the volume was reduced by 98% to 9 ml 19 months later. On a later control, the volume was further reduced.

We regret the absence of a control group with cytologic and biochemical tests before and after catheterization of the liver cysts, but without the use of a sclerosing agent. Infrequently, we catheterized the liver cysts and evacuated them without sclerotherapy in order to determine whether the symptoms were related to the cyst or not. But at our institution it would have taken more than 5 years to get 5 patients into such a control group. In our opinion the catheterization of volunteers would be too problematic in terms of practicality and ethics.

In conclusion, it is not possible from the cytologic and biochemical analysis alone to claim that the ethanol-induced devitalization of the cystic epithelium is complete and that no remnants of vital cyst wall epithelium remain. However, our results favor the hypothesis that the post-sclerotherapy fluid production is caused by an inflammatory reaction in a cystic wall derived of vital epithelium. This time-limited inflammation may explain why repeated sclerotherapy sessions are unnecessary even if transitory re-accumulation of fluid is observed in liver cysts following ethanol sclerotherapy.

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