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Aberrant expression of cytokeratin 7 in perivenular hepatocytes correlates with a cholestatic chemistry profile in patients with heart failure

Rish K Pai and John A Hart[1]

Department of Pathology, The University of Chicago Medical Center, Chicago, IL, USA

A cholestatic liver chemistry profile (elevations in alkaline phosphatase and bilirubin) is commonly encountered in patients with hepatic venous outflow obstruction due to right heart failure. Liver biopsies from these patients demonstrate varying degrees of sinusoidal dilatation, red blood cell extravasation and sinusoidal congestion. Recently, a bile ductular reaction mimicking biliary tract disease has been identified in ~50% of patients with venous outflow obstruction possibly explaining the cholestatic profile encountered in these patients. In this study, we evaluated the liver biopsies from 22 patients with heart failure. Marked sinusoidal dilatation involving zones 2 and 3 was observed in 15 patients. Similar to a previous study, 7 of 22 patients had histologic evidence of a mild ductular reaction. Cytokeratin 7 immunohistochemistry revealed a mild ductular reaction in an additional two cases. Strikingly, CK7 was aberrantly expressed in perivenular hepatocytes in 20 of 22 cases. Perivenular CK7 immunoreactivity was focal in most cases; however, in five cases it was quite extensive and extended into zone 2. There was no significant association between marked sinusoidal dilatation and extensive perivenular CK7 positivity. Extensive perivenular CK7 positivity was significantly associated with both elevated bilirubin, as well as the presence of fibrosis. However, a ductular reaction was not associated with a cholestatic liver chemistry profile.
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Hepatic venous outflow obstruction caused by heart failure is characterized by the presence of sinusoidal dilatation, congestion, red blood cell extravasation in the space of Disse, and hepatocyte atrophy. These changes are most severe in zone 3. With persistent obstruction there is hepatocyte dropout and pericellular fibrosis. Rarely more extensive fibrosis (cardiac sclerosis) can be seen,[1–3] which warrants consideration of a combined heart and liver transplantation.

It is interesting that the patients with outflow obstruction often have cholestatic liver chemistry tests that are at odds with the histological features. Elevations in alkalaine phosphatase and bilirubin two to five times the upper limit of normal are not uncommon. Kakar *et al* recently described portal changes in the liver biopsies of patients with hepatic venous outflow obstruction due to right heart failure, Budd–Chiari syndrome, and veno-occlusive disease.[4] They found that nearly half of the patients had evidence of a ductular reaction; however, none of the patients had evidence of biliary tract disease. They argued that such changes could account for the liver chemistry abnormalities. However, many patients without histological evidence of a ductular reaction have a cholestatic liver chemistry profile. In this study we analyzed the histologic and biochemical features of 22 patients with congestive heart failure undergoing liver biopsy for the evaluation of either heart transplantation or combined heart/liver

Correspondence: Dr RK Pai, MD, PhD (Current address): Department of Anatomic Pathology, Cleveland Clinic Foundation, 9500 Euclid Avenue, Mail Code L56, Cleveland, OH 63110-1093, USA.
E-mail: pair@ccf.org
[1]Current address: Dr JA Hart, Department of Pathology, The University of Chicago, 5841 S. Maryland Avenue, E-607D, MC 6101, Chicago, IL 60637, USA.
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transplantation. We show that zone 3 hepatocytes aberrantly express cytokeratin 7 (CK 7), an intermediate filament predominately expressed in biliary epithelium, in the majority of cases. Furthermore, extensive perivenular CK7 positivity, not a ductular reaction, correlated with statistically significant elevations in total bilirubin, as well as the presence of fibrosis.

Materials and methods

Case Selection

Our surgical pathology files were searched for liver biopsies with a diagnosis of 'outflow obstruction' between 2002 and 2009. This search yielded a total of 89 liver biopsies. From this pool of biopsies only patients with a clinical diagnosis of 'heart failure' were included. Patients with other concomitant diseases (chronic hepatitis, biliary tract disease, liver transplantation, etc) were excluded. Patients with other causes of outflow obstruction were also excluded. These criteria resulted in the selection of 22 study patients with heart failure and outflow obstruction. The majority of these patients were undergoing a liver biopsy as part of the evaluation for a heart transplant.

Histological Evaluation

All biopsies were reviewed and the following parameters were scored: sinusoidal dilatation (zone 3 only (+), zones 2 and 3 (++), and zones 1–3 (+++)), fibrosis (pericellular, perivenular portal, and bridging), portal inflammation (absent, mild, and moderate), ductular reaction (absent, mild, and extensive), and bile duct damage (absent or present).

Clinical and Laboratory Data

Age and gender were recorded. In addition total bilirubin, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase at the time of biopsy were recorded.

Immunohistochemistry for CK7 and Scoring

Sections were deparaffinized and the antigen was retrieved by the heating slides in a microwave in citrate buffer. Endogenous peroxidase was blocked by incubation with 3% hydrogen peroxidase. Slides were then incubated with 0.3% bovine serum albumin–Tris buffered saline (TBS) to reduce nonspecific background staining. Anti-CK7 antibody (Dako, Carpinteria, CA, USA) was added followed by incubation with biotinylated secondary antibody, peroxidase-conjugated streptavidin and diaminobenzidine chromogen (Dako) for visualization. This incubation was followed by TBS rinses and then counterstained with hematoxylin. The degree of CK7 positivity was scored as + (focal zone 3), ++ (diffuse zone 3), and +++ (zones 2 and 3). In addition, the portal areas were examined for a subtle ductular reaction not identified on hematoxylin and eosin stains.

Statistical Analysis

We performed the nonparametric Mann–Whitney test, as well as Fisher's exact test and the statistical significance was set at $P<0.05$ (SPSS V13, Chicago, IL, USA).

Results

Clinical and Histological Findings

A total of 22 patients with a histological diagnosis of outflow obstruction and a clinical diagnosis of heart failure were analyzed (Table 1). A total of 14 patients (64%) had elevations in alkaline phosphatase (normal 30–120 U/l) and 15 (68%) had elevations in total bilirubin (normal $\leq$1.0 mg per 100 ml). A total of 10 patients had elevations in both bilirubin and alkaline phosphatase. aspartate aminotransferase and alanine aminotransferase levels were below 60 IU/ml in all but two cases. All patients were negative for viral hepatitis.

The histological features are summarized in Table 1. All patients had at least sinusoidal dilatation in zone 3, whereas 15 (68%) had marked sinusoidal dilatation (zones 1–3 (+++) or zones 2–3 (++)). Four patients had zone 3 pericellular fibrosis and an additional three patients had more extensive zone 3 fibrosis with focal central vein obliteration. Only one patient had focal bridging fibrosis and no patients had cirrhosis. Similar to Kakar *et al*, 32% of patients had a mild ductular reaction (Figure 1a); however, no significant bile duct injury was seen. Portal inflammation was present in the majority of cases, but was mild. One patient had focal ballooning degeneration and Mallory hyaline related to amiodarone toxicity. One case had a single-portal-based epitheliod granuloma that was not associated with a bile duct and is of unknown significance.

CK7 Immunohistochemistry

A CK7 immunohistochemical stain was performed on all 22 liver biopsies. A CK 7 stain highlighted a subtle ductular reaction in an additional two cases (Figure 1b). It is surprising that the perivenular hepatocytes aberrantly expressed CK7 in the majority of cases. In 14 cases aberrant CK7 expression was limited to zone 3 in a diffuse (++, $n=3$) or focal (+, $n=11$) manner (Figure 2). In five cases there was marked CK7 expression (+++) that extended beyond zone 3 and into zone 2 (Figure 3).

Table 1 Clinical and histological characteristics of patients with heart failure

Patient	*Total bilirubin (mg per 100 ml, normal range 0.1–1.0 mg per 100 ml)*	*Alkaline phosphatase (U/l, normal range 30–120 U/l)*	*AST (U/l, normal range 5–40 U/l)*	*ALT (U/l, normal range 7–56 U/l)*	*Sinusoidal dilatation*	*Portal inflammation*	*Ductular reaction*	*Fibrosis*	*CK7 (hepatocytes)*	*CK7 (periportal ductules)*
1	0.9	448	13	20	+++	Mild	—	Zone 3 with focal bridging	+++	—
2	1.3	202	30	55	+++	Mild	Mild	Zone 3	+	Mild
3	1.5	160	33	47	+++	—	—	None	+	Mild
4	8.0	127	25	31	+++	Mild	—	None	+++	—
5	5.7	97	26	37	+++	Mild	—	Zone 3 with focal central vein obliteration	+++	—
6	3.1	63	33	34	+++	—	Mild	None	+	Mild
7	1.3	504	29	25	++	—	—	Zone 3	+	—
8	2.1	214	53	53	++	—	—	None	+	—
9	2.5	169	13	23	++	Mild	—	Zone 3	+++	—
10	2.1	178	37	57	++	Mild	Mild	None	+	Mild
11	0.5	175	42	37	++	—	—	None	+	—
12	2.9	125	14	26	++	Mild	Mild	Zone 3 with focal central vein obliteration	+++	Mild
13	0.8	119	27	33	++	—	Mild	None	++	Mild
14	3.1	57	34	37	++	—	—	None	++	—
15	1.2	35	28	48	++	—	—	None	+	—
16	0.6	249	19	33	+	Mild	—	None	+	—
17	0.4	204	20	21	+	Mild	Mild	Zone 3	++	Mild
18	1.8	154	262	119	+	Mild	—	Zone 3	+	Mild
19	5.9	135	20	50	+	Mild	Mild	None	+	Mild
20	0.2	117	15	17	+	Mild	—	None	—	—
21	0.6	104	85	36	+	—	—	None	—	Mild
22	1.3	77	24	26	+	Mild	—	None	+	—

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Perivenular expression of CK7 correlated with the presence of fibrosis as four of eight cases with fibrosis had marked CK7 expression (+++) compared with only one of 14 cases without fibrosis ($P=0.04$). There was no significant correlation between the degree of hepatocyte CK7 expression and sinusoidal dilatation.

Correlation with Cholestatic Chemistry Profile

The histologic and immunohistochemical features that correlate with a cholestatic chemistry profile were determined (Table 2). Elevated total bilirubin and alkaline phosphatase were seen in patients with extensive sinusoidal dilatation; however, this did not reach statistical significance. Extensive (+++) perivenular hepatocyte CK7 expression correlated with statistically significant elevations in total bilirubin, but not alkaline phosphatase. In addition, patients with fibrosis had statistically significant elevations in alkaline phosphatase compared with those without this feature. In contrast, the presence of a ductular reaction did not correlate with an increased total bilirubin or alkaline phosphatase.

Discussion

Bile production by hepatocytes is a complex and tightly regulated process. Bile produced by hepatocytes is exported into the canalicular system where it is transported through canals of Hering and bile ductules to interlobular bile ducts and finally to large septal bile ducts. Numerous disease processes and medications are known to disrupt this process resulting in a cholestatic pattern of liver chemistry tests. One cause of a cholestatic chemistry profile that can result in a misdiagnosis of a biliary tract abnormality is a disease that affects venous outflow. Patients with outflow obstruction due to diverse causes, such as sinusoidal obstruction syndrome, Budd–Chiari syndrome and heart failure, often have an elevated alkaline phosphatase, as well as total bilirubin.

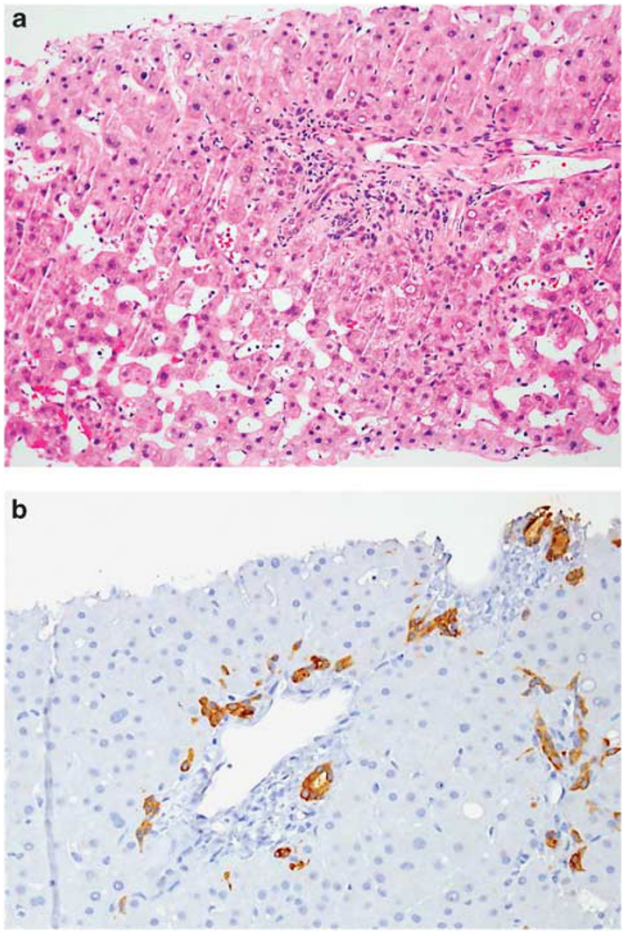

Figure 1 (**a**) A mild ductular reaction is present in this portal tract (hematoxylin and eosin (H&E), ×200). (**b**) A definitive ductular reaction was not identified in this portal tract on H&E; however, a cytokeratin 7 immunohistochemical stain highlighted a mild ductular reaction (×100).

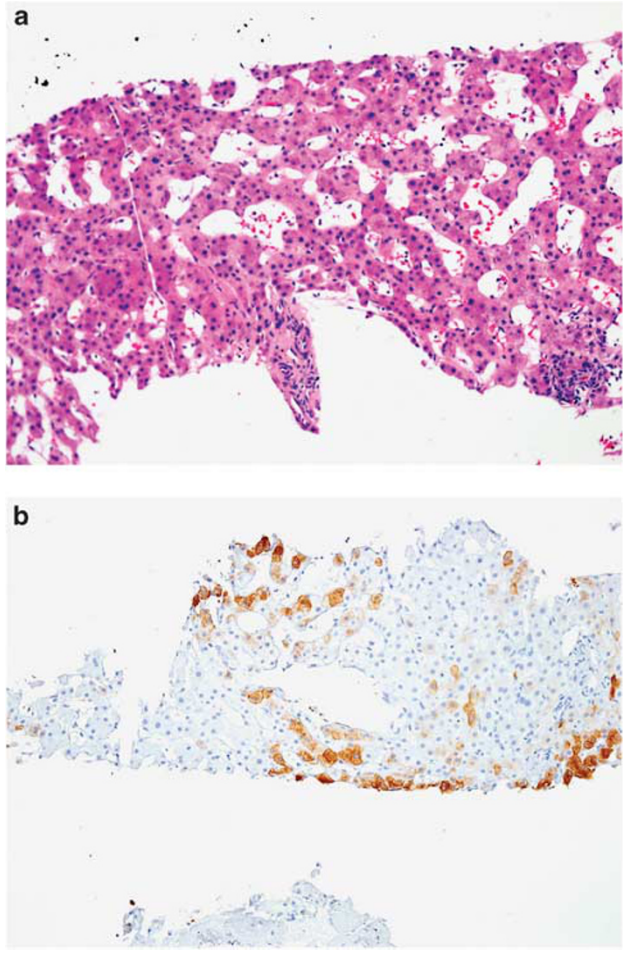

Figure 2 (**a**) Mild sinusoidal dilatation in zone 3 is seen in this case (hematoxylin and eosin, ×200). (**b**) Perivenular cytokeratin 7 immunoreactivity is identified (×200).

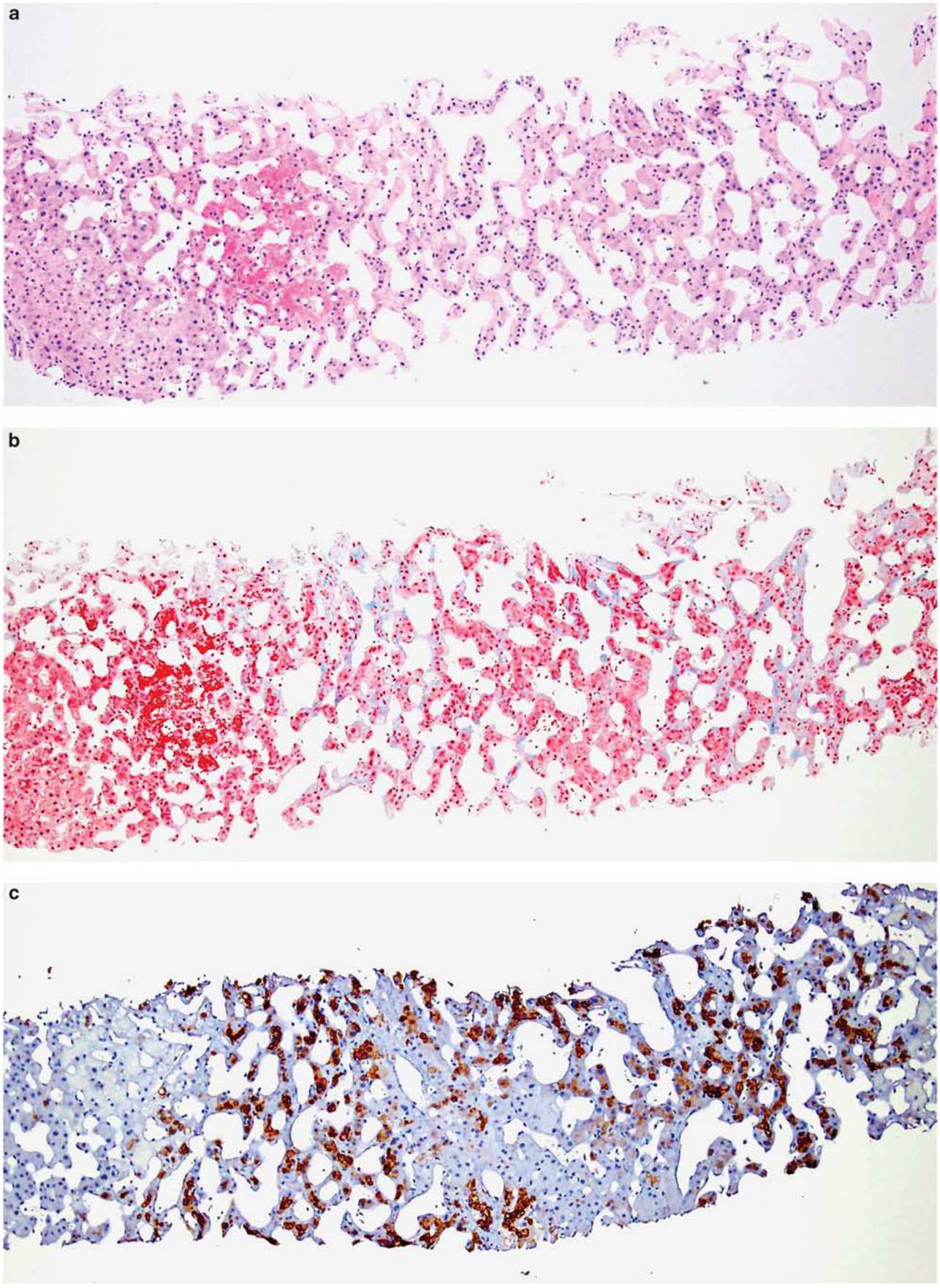
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Figure 3 (**a**) Marked sinusoidal dilatation, hepatic cord atrophy, and congestion is identified in this case (hematoxylin and eosin, ×40). (**b**) Mild pericellular fibrosis is seen (trichrome, ×40). (**c**) Cytokeratin 7 immunohistochemistry highlights extensive aberrant expression in hepatocytes in zones 2 and 3 (×40).

Table 2 Correlation with cholestatic liver chemistry tests

Category	N	*Total bilirubin (mg per 100 ml)*	*Alkaline phosphatase (U per 100 ml)*	*AST*	*ALT*
All patients	22	2.2	169	40	39
Sinusoidal dilatation					
Zone 3 (+)	7	1.5	149	64	43
Zone 2–3 (++)	9	1.8	175	31	38
Zones 1–3 (+++)	6	3.4	183	27	37
CK7 positivity					
Zone 3 (focal (+) and diffuse (++))	14	1.9	169	30	40
Diffuse Zones 2–3 (+++)	5	4.0*	193	18	27
Ductular reaction	9	2.1	147	26	39
Fibrosis	8	2.1	238**	51	41

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.
Value is significantly different from those cases without this feature, $^{*}P=0.03$, $^{**}P=0.04$.

In this study, we show that 19 of 22 patients with heart failure had elevations in either alkaline phosphatase or total bilirubin. Despite these elevations, liver biopsies did not show canalicular, hepatocellular or ductular cholestasis. Kakar *et al* demonstrate a bile ductular reaction at the periphery of portal tracts.[4] The ductular reaction was quite mild in many cases. We confirmed this finding in our study population as 9 of 22 (41%) either had histological (seven) or immunohistochemical (two) evidence of a ductular reaction. As bile ductules have a poorly formed basement membrane,[5] bile and alkaline phosphatase could potentially leak into the sinusoids resulting in a cholestatic chemistry profile. However, many patients with biochemical cholestasis did not demonstrate a ductular reaction on biopsy. Furthermore, the presence of a ductular reaction did not correlate with elevated bilirubin or alkaline phosphatase in this study.

In addition to being expressed in bile ducts, bile ductules, and hepatic progenitor cells, CK7 is also expressed by hepatocytes in cholestatic conditions, such as biliary obstruction, primary biliary cirrhosis, primary sclerosing cholangitis, and chronic allograft rejection.[6–8] Expression of CK7 in these conditions is though to represent a metaplastic phenomenon in which these cells take on a hybrid phenotype between a hepatocyte and cholangiocyte. Thus, it was quite surprising that CK7 was expressed in perivenular hepatocytes in the majority of patients with heart failure in this study. In five patients the CK7 expression was quite striking and extended into zone 2. This patient cohort had the most striking cholestatic chemistry profile with statistically significant elevations in total bilirubin. In addition there was a statistically significant association between hepatocyte CK7 expression and fibrosis.

Damage to the hepatic artery is a well-known cause of injury to the biliary tree, as this vessel is the main source of blood supply.[9] Obstruction of the portal vein inflow can also cause bile duct injury, so called 'portal biliopathy'.[10] It is unclear how obstructing venous outflow results in disruption of bile flow and a cholestatic chemistry profile. It is possible that the increased sinusoidal pressure causes injury to the canalicular system because of compression of the hepatic plates. As bile salts are toxic, perivenular hepatocytes could begin to express biliary CKs as a protective response.

Cholangiocytes are also well-known contributors to the development of fibrosis. A bile ductular reaction has been implicated in the development of fibrosis in primary diseases of the biliary tract, as well as hepatitis C and steatohepatitis.[11,12] Cholangiocytes can undergo epithelial-to-mesenchymal transition and produce collagens.[13] They also stimulate other cell types in the liver, in particular the stellate cell and periductal myofibroblasts, to produce extracellular matrix.[14–16] Given the positive association between hepatocyte CK7 expression and fibrosis in this study, it is possible that the expression of CK7 by hepatocytes may contribute to the collagen deposition observed in patients with severe heart failure.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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