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Dear Editor

Accumulation of copper and copper binding protein in periportal hepatocytes is generally considered to be an indicator of chronic cholestasis and a sign of chronic biliary pathology, particularly at an early stage when bilirubinostasis is lacking (1). Rhodanine and Orcein or Victoria blue staining methods detect copper and copper binding protein respectively. Cytokeratin 7 expression by periportal hepatocytes or hepatocyte-like cells (intermediate hepatobiliary cells) (2) is also considered an early marker of chronic cholestasis (1). It is referred to as “periportal Ker7” in the rest of this letter.

It is unknown, to our knowledge, how the stains for copper and copper-binding protein and periportal Ker7, perform reciprocally in liver biopsies from patients with biliary disorders, and whether they distribute uniformly around the same portal tracts. We have therefore selected 12 core needle biopsy specimens obtained from 12 patients who underwent liver biopsy due to a clinical suspicion of a biliary disorder and/or who demonstrated a biliary pattern of injury histologically (see table 1). Additional selection criteria included: each sample had to have four sections routinely stained for orcein, cytokeratin 7, and rhodanine in the liver histopathology laboratory of the Institute of Liver Studies at King’s College Hospital; each sample had one or more portal tracts which could be identified in all three stained sections. Using an Olympus BX51 light microscope, each portal tract visible in all three sections was marked, and each stain in the

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hepatocytes around it assessed as follows: Negative: no visible granules with rhodanine or orcein stain, no periportal Ker7; Positive: presence of rhodanine or orcein positive granules ranging from a few granules identified after careful search at high magnification (400x), or very occasional periportal Ker7, to granules obvious at lower magnifications and numerous periportal Ker7.

These 12 biopsy samples contained a total number of 82 portal tracts which could be identified in all four sections (range 1-14, average 6.8). When the stains were assessed cumulatively in each biopsy (i.e. regardless of their reciprocal relationship in each periportal region), four biopsies showed positive cytokeratin and orcein stains, three biopsies showed positive cytokeratin 7 stain only, two biopsies showed positive orcein and rhodanine (i.e. negative cytokeratin 7 stain) and three biopsies showed all stains to be positive. In other words, cytokeratin 7, orcein and rhodanine stains were positive in 10, 9 and 5 of twelve samples. None of the samples showed orcein or rhodanine stains in isolation.

When each stain was considered in the 82 periportal regions cumulatively and regardless of the performance of the other stains, cytokeratin 7 stain was present around 26 portal tracts (32%), orcein positivity was present around 18 (22%) portal tracts, and rhodanine positivity around 11 (13%) portal tracts.

The reciprocal stain combinations are illustrated in figure 1. Cytokeratin 7 stain alone was present around 13 portal tracts (16%), coexistent orcein and cytokeratin 7 stains around 6 portal tracts (7%), coexistent orcein and rhodanine stains around 4 portal tracts (5%), and orcein stain alone around 1 portal tract (1%). The number of periportal regions with no positive stains was 51 (62%). All stains were positive around 7 portal tracts (9%)(an example is shown in figure 2).

Our observations indicate that the distribution of periportal histological signs of chronic cholestasis in chronic cholangiopathies is patchy and that periportal regions are affected in a heterogeneous fashion, with about half of them showing no histological signs of chronic cholestasis in our series. The stains are not reciprocally linked, as only in a minority (9%) of periportal regions all stains were positive. Adding cytokeratin 7 to a routine set of histochemical stains including orcein, should ensure that all signs of chronic cholestasis are detected in biopsies from patients with chronic cholangiopathies. Due to the patchy distribution of the stain positivity in our series, it is hard to conclude whether periportal Ker7 positivity precedes the accumulation of copper and copper-binding protein.

Reference

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2. Scheuer's Liver Biopsy Interpretation, 9th Edition, 2015. Elsevier

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Figure 1

Table 1. Patient's details

Pt	Age	Sex	Clinical picture	Histology
1	57	M	5 years post-liver transplant for primary sclerosing cholangitis (PSC). Cholangiopathy on MRCP ? recurrence ALP 186 (30-130 IU/L) GGT 326 (1-55 IU/L), bilirubin 5 (3-20 umol/L)	Lymphocytic cholangitis of interlobular bile ducts and ductular reaction
2	8	F	Ulcerative colitis ? primary sclerosing cholangitis ALP 228 (156-386 IU/L) GGT 103 (1-55 IU/L), bilirubin 4 (3-20 umol/L)	Bridging fibrosis with peribiliary sclerosis and ductular reaction
3	4	F	Inflammatory bowel disease ? PSC ALP 228 (134-386 IU/L) GGT 76 (1-55 IU/L), bilirubin 3 (3-20 umol/L)	Bridging fibrosis with peribiliary sclerosis and ductular reaction
4	35	M	? biliary disorder ALP 299 (30-130 IU/L) GGT 681 (1-55 IU/L), bilirubin 8 (3-20 umol/L)	Mild portal expansion and ductular reaction
5	43	F	Cholestatic liver biochemistry	Mild portal fibrosis with cholangitis of interlobular bile ducts and focal peribiliary sclerosis
6	14	F	Family history of IBD and PSC. ALP 289 (62-209 IU/L) GGT 182 (1-55 IU/L), bilirubin 7 (3-20 umol/L)	Attenuation of interlobular bile ducts and ductular reaction
7	31	F	? IgG4 related cholangiopathy ? Primary biliary cholangitis (PBC) ALP 225 (30-130 IU/L) GGT 269 (1-55 IU/L), bilirubin 6 (3-20 umol/L)	Bridging fibrosis , portal inflammation with granulomatous component
8	24	F	20 years post-liver transplant for acute liver failure. ALP 112, GGT 148 (6-42 IU/L), bilirubin 6 (3-20 umol/L)	Cholangitis of part of a large bile duct

9	67	F	? autoimmune hepatitis ? PBC	Granulomatous cholangitis in keeping with PBC
10	54	M	? sarcoid ALP 197 (30-130 IU/L) GGT 1063 (1-55 IU/L), bilirubin 8 (3-20 umol/L)	Bridging fibrosis, ductular reaction, injury to interlobular bile ducts and peribiliary fibrosis
11	36	F	Cholestatic biochemical profile ? cause ALP 757 (30-130 IU/L) GGT 538 (1-55 IU/L), bilirubin 5 (3-20 umol/L)	Mild ductular reaction. No significant fibrosis.
12	59	F	10 months post-liver transplant for PBC. Anastomotic biliary stricture ALP 409 GGT 1481, bilirubin 6	Mild ductular reaction

Cytokeratin 7 , orcein and rhodanine stain in 82 portal tracts from 12 core needle biopsies

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