



Immunohistochemical evaluation of hepatic progenitor cells in different types of feline liver diseases

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ABSTRACT. Hepatic progenitor cells are periportal resident cells capable of differentiating into mature hepatocytes or cholangiocytes to ensure hepatic regeneration. This reaction is termed a ductular reaction. In the present study, regenerative response of the feline liver to different hepatic diseases was investigated immunohistochemically. Regeneration of the liver through hepatocellular replication and proliferation of progenitor cell compartment were comparatively evaluated. Histological and immunohistochemical stainings were conducted on feline liver samples (n=40) representing various hepatobiliary diseases. Cytokeratin (CK) 7, CK19, Proliferating cell nuclear antigen (PCNA), Ki67, and Human hepatocyte marker 1 (Hep Par-1) were used. The presence of progenitor cells within feline livers was proved, both as passive cells in normal liver and as active cells (ductular reaction) in hepatic lesions. CK7 was found to be a suitable antibody for immunohistochemically detecting feline progenitor cells. In acute events, regeneration was predominantly shaped by the division of hepatocytes. In chronic events and severe acute events, hepatocytes lost their ability to divide and regeneration mainly occurred through progenitor cells. Location of the ductular reaction varied between different hepatic diseases. Parenchymal ductular reaction was detected in fulminant hepatitis, chronic hepatitis, hepatocellular lipidosis and metastatic lymphoma, whereas septal ductular reaction was detected in chronic hepatitis and metastatic lymphoma. Ductular reaction exhibited positive staining for Hep Par-1 in chronic and severe acute events. This study indicates the major role played by hepatic progenitor cells in regeneration of the feline liver. Moreover, it shows how the activation pattern of ductular reaction varies according to the hepatobiliary disease type.

KEY WORDS: ductular reaction, feline, hepatic progenitor cell, immunohistochemistry, regeneration

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The repair-related activities of the liver involve balanced reactions between parenchymal regeneration and fibrosis. Any imbalances in these processes can lead to cirrhosis, tumor formation or acute organ failure [20]. The liver is an organ that exhibits significant regenerative ability. Indeed, mild injury can be completely healed through hepatocellular regeneration followed by the replication of the bile duct epithelium, Kupffer cells, stellate cells, and sinusoidal endothelial cells [3, 12]. As hepatic regeneration can involve the division and proliferation of hepatocytes, it can also be shaped by the differentiation of the hepatic progenitor cells into either hepatocytes or biliary cells [3].

Advances in hepatology have indicated that hepatocellular replication is weak or absent in cases of severe or chronic liver disease. In such cases, the regeneration of the hepatocytes occurs through the progenitor cell population of the liver [1, 6, 7, 18, 21]. The progenitor cells, which are also known as the local stem cells of the liver, are located in the canals of Hering in the periportal zone of the lobule. In a normal liver, single resident basophilic progenitor cells can be visualized [6, 8]. When activated, these cells proliferate and attempt to regenerate the damaged liver. The resultant reaction is defined as a ductular reaction, and it compromises single active progenitor cells, small bile ductular structures that usually lack distinguishable lumen, and intermediate-sized hepatobiliary cells [3–5, 19].

The hepatic progenitor cells can be histologically differentiated during activation on the basis of their morphology (ductular reaction). According to previous articles, biliary antibodies such as Cytokeratin (CK) 7 and CK19 can be used to distinguish these cells immunohistochemically [2, 14, 19, 21]. When the progenitor cells start to differentiate into hepatocytes, they gradually lose their ability to express biliary antibodies and gain a hepatocellular phenotype that can be visualized using hepatocellular markers such as Human hepatocyte marker 1 (Hep Par-1) or albumin [6, 18]. In addition, hepatocellular replication can be

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immunohistochemically visualized using proliferation markers such as Proliferating cell nuclear antigen (PCNA) and Ki67 [12, 13].

Research interest in the hepatic progenitor cells within canine and feline species has increased in recent years. However, although the hepatic progenitor cells found in canine livers have been described in detail [1, 6–8, 10, 18, 24], only a few studies have been conducted in feline species [5, 8, 14, 15, 21]. To date, there has been only one comparative evaluation of both hepatic regenerative pathways in different feline liver diseases [5].

The present study aimed to comparatively evaluate the regenerative pathways of the feline liver in various hepatobiliary diseases. Moreover, the study also sought to consecutively immunohistochemically characterize the contribution and pattern of the reaction of hepatic progenitor cell compartment in relation to the regeneration of a damaged liver.

MATERIALS AND METHODS

The liver samples used in this study were obtained from cats submitted to the Pathology Department of Ankara University, Faculty of Veterinary Medicine for routine necropsy. Ethical approval for the study (protocol 83 no-2016-23-197) was granted by the Ethical Committee of Ankara University. A total of 40 samples were included in this study, representing the most common hepatic diseases found in feline species submitted to the faculty between April 2017 and September 2018. More specifically, the disease samples comprised hepatic displacement (n=2), passive congestion (n=3), acute hepatitis (n=3), fulminant hepatitis (n=2), subacute hepatitis (n=6), chronic hepatitis (n=2), metastatic lymphoma (n=3), hepatocellular adenoma (n=1), lymphocytic cholangitis/cholangiohepatitis (n=8), hepatocellular lipidosis (n=9), congenital cystic disease (n=1) (Table 1).

The liver samples were fixed in 10% formalin, embedded in paraffin and sections (4 µm) were routinely stained with Harris's hematoxylin and eosin (H&E). The histopathological evaluation was based on the WSAVA (World Small Animal Veterinary Association, Liver Standardization Group) histological criteria. The immunohistochemical staining was performed using CK7, CK19, PCNA, Ki67 and Hep Par-1 antibodies. Antibody specifications and processing characteristics are summarized in Table 2. The sections mounted on charged glass slides were deparaffinized and then rehydrated with graded alcohols and xylol. Heat-induced antigen retrieval method was used (Table 2). Sections were then incubated in 3% H₂O₂ for 15 min to block the endogenous peroxidase activity. To block the background staining, the slides were incubated with blocking serum (Ultra V block, Thermo Fisher Scientific, Fremont, CA, USA) for 35 min at room temperature.

As liver cells are known to be rich in endogenous biotin, and because the citrate buffer used for antigen retrieval has been found to increase the reactivity of endogenous biotin in tissues, the slides were subjected to endogenous biotin blocking using egg white and milk powder (for the CK7, CK19, PCNA and Ki67 antibodies) [9, 11]. Subsequently, the sections were incubated with the primary antibodies for 1 hr at 37°C. For CK7, CK19, PCNA and Ki67, the Ultravision Quanto Detection System Horseradish Peroxidase (HRP) was used for secondary labeling (Thermo Fisher Scientific, catalog no. TL-060-QHL, 35 min each) and the reaction was visualized using aminoethyl carbazole substrate AEC (Thermo Fisher Scientific, TA-125-HA). For Hep Par-1 antibody, the Bond Polymer Refine Detection DAB kit (Leica Microsystems, catalog no. DS9800, New Castle, UK) was used for secondary labeling, while color was developed using 3'-Diaminobenzidine tetrahydrochloride hydrate (DAB). Sections stained with AEC were counterstained with Gill's hematoxylin and then mounted with aqueous mounting medium. Slides stained with DAB were counterstained with Mayer's hematoxylin, dehydrated, and then mounted in entellan mounting medium. The bile ducts of a normal liver section were examined as an internal positive control for CK7 and CK19 antibodies. For PCNA and Ki67 antibodies, a feline normal lymph node section was used as a positive control, while a normal liver section was also stained. For Hep Par-1 antibody, feline normal liver slide was stained as a positive control. Moreover, as negative controls, the primary antibodies were replaced with an equivalent amount of mouse IgG isotype serum.

Immunopositivity for CK7, CK19 and Hep Par-1 were assessed semi-quantitatively in four different histological areas of the liver (portal, periportal, parenchymal and septal). Five high-power fields (HPF, ×400 magnification) of each separate area were assessed for positively staining hepatocytes and biliary cells and a score on a 0–3 positive scale was given (0: no positive cells, +: mild number of positive cells, ++: moderate number of positive cells, +++: prominent number of positive cells). Mitotic activity of hepatocytes assessed with PCNA and Ki67 staining was determined by counting positive hepatocytes on 10 HPF (×40). A mean of 150 hepatocyte drops in each HPF therefore percentages for each slide were determined and a score was consequently given (0: no positivity; 1: low positivity 1–3%; 2: moderate positivity 3–5%; 3: high positivity >5%).

RESULTS

Feline normal liver section, used as positive control, showed strong cytoplasmic positive staining for CK7 in the epithelium of the portal bile ducts and few single oval shaped cells in the periportal areas. In various feline hepatic diseases, an increase in the number of CK7 positive cells in portal, periportal, parenchymal or septal areas was observed, and this was evaluated as ductular reaction. The intensity and localization of CK7 positive cells were scored for each case (Table 1).

In acute/subacute hepatitis and acute hepatic diseases such as hepatic displacement and acute passive congestion, CK7 immunohistochemical staining revealed the absence or mild presence of increased CK7 positive cells (Fig. 1A). These cells were mainly localized in the portal and periportal areas and appeared as few positively stained ductules lacking a lumen and single oval cells. Expansion of these oval cells toward the parenchyma was observed in only two cases (acute hepatitis 'case 6' and subacute hepatitis 'case 13'). It is worth mentioning that these positive cells were specially localized around the intensively necrotic areas.

In fulminant hepatitis, a prominent portal and periportal staining of newly formed biliary structures was detected surrounding

Table 1. Semiquantitative immunohistochemical scoring of Cytokeratin (CK) 7 and proliferating cell nuclear antigen (PCNA) and their distribution in feline hepatic diseases

| Case No. | Breed | Age [§] | Sex [†] | Diagnosis | CK7 | | | | PCNA |
|----------|-------------------|------------------|------------------|---|--------|------------|-------------|--------|------|
| | | | | | Portal | Periportal | Parenchymal | Septal | |
| 1 | Tabby | 5 m | F | Hepatic displacement | + | - | - | - | + |
| 2 | Crossbreed | 2 m | F | Hepatic displacement | ++ | + | - | - | +++ |
| 3 | Crossbreed | 4 m | F | Passive congestion | - | - | - | - | + |
| 4 | Siamese | 1 y | M | Passive congestion | + | - | - | - | +++ |
| 5 | Tabby | 5 y | M | Passive congestion | + | - | - | - | ++ |
| 6 | Orange tabby | 8 m | F | Acute hepatitis | ++ | ++ | + | - | ++ |
| 7 | Tabby | 1 y 5 m | F | Acute hepatitis | - | + | - | - | ++ |
| 8 | Persian | 8 m | F | Acute hepatitis | + | + | - | - | +++ |
| 9 | Tabby | 6 y | M | Fulminant hepatitis | +++ | +++ | - | - | + |
| 10 | Crossbreed | 1 y | M | Fulminant hepatitis | +++ | +++ | ++ | - | + |
| 11 | * | 3 m | F | Subacute hepatitis | + | - | - | - | +++ |
| 12 | Orange tabby | * | F | Subacute hepatitis | + | - | - | - | - |
| 13 | Tabby | 5 y | M | Subacute hepatitis | ++ | ++ | + | - | +++ |
| 14 | Crossbreed | 6 m | F | Subacute hepatitis | ++ | + | - | - | ++ |
| 15 | Crossbreed | 4 m | F | Subacute hepatitis | + | - | - | - | ++ |
| 16 | Tabby | 2 m | F | Subacute hepatitis | + | + | - | - | - |
| 17 | British shorthair | 6 m | M | Chronic hepatitis | ++ | ++ | - | - | + |
| 18 | * | 16 y | M | Chronic hepatitis | ++ | + | ++ | + | + |
| 19 | Bombay | 10 y | M | Metastatic lymphoma | +++ | +++ | ++ | ++ | + |
| 20 | Orange tabby | 11 y | M | Metastatic lymphoma | + | + | - | - | + |
| 21 | Tabby | 2 y | M | Metastatic lymphoma | ++ | ++ | ++ | ++ | + |
| 22 | Orange tabby | 18 y | F | Hepatocellular adenoma Lymphocytic cholangitis | + | ++ | - | - | +++ |
| 23 | Tabby | 2 y | M | Lymphocytic cholangiohepatitis | + | + | - | - | + |
| 24 | * | 5 m | M | Lymphocytic cholangiohepatitis | + | + | - | - | - |
| 25 | Crossbreed | 3 y | M | Lymphocytic cholangiohepatitis | + | + | - | - | + |
| 26 | Tabby | 2 y | F | Lymphocytic cholangiohepatitis | ++ | ++ | + | - | ++ |
| 27 | Crossbreed | 1 y | F | Lymphocytic cholangiohepatitis | ++ | ++ | - | - | + |
| 28 | Scottish fold | 2 m | F | Lymphocytic cholangiohepatitis | + | + | - | - | + |
| 29 | Tabby | 12 y | M | Lymphocytic cholangitis | ++ | ++ | + | - | + |
| 30 | Crossbreed | 2 m | F | Lymphocytic cholangitis | + | + | - | - | + |
| 31 | British shorthair | 1 m | F | Hepatocellular lipidosis | + | + | - | - | + |
| 32 | Tabby | 7 y | F | Hepatocellular lipidosis | + | ++ | + | - | + |
| 33 | Crossbreed | 22 y | M | Hepatocellular lipidosis | ++ | ++ | ++ | - | ++ |
| 34 | Himalayan | 8 y | F | Hepatocellular lipidosis | ++ | ++ | + | - | ++ |
| 35 | Crossbreed | 1 y | M | Hepatocellular lipidosis | + | + | - | - | ++ |
| 36 | Crossbreed | 6 m | M | Hepatocellular lipidosis | + | - | - | - | + |
| 37 | Tabby | * | M | Hepatocellular lipidosis | + | + | + | - | ++ |
| 38 | Scottish fold | 2 m | F | Hepatocellular lipidosis | + | + | - | - | + |
| 39 | Van cat | 1 y | F | Hepatocellular lipidosis | ++ | ++ | + | - | ++ |
| 40 | Persian | 3 y | F | Congenital cystic disease | +++ | ++ | + | ++ | + |

[§] m=month; y=year. [†]M=male; F=female. *Unknown. -: no positive cells; +, mild number of positive cells; ++, moderate number of positive cells; +++, prominent number of positive cells.

portal inflammatory reactions (Fig. 1B). These structures also radiated deep into the parenchyma, forming a moderate parenchymal CK7 staining and were accompanied by intermediate cells featuring membranous CK7 staining (Fig. 2). Besides, hepatocytes in fulminant hepatitis cases slightly stained positive with CK7.

In chronic hepatitis, moderately increased CK7 positive ductular structures were located in portal and periportal areas of both samples with septal and parenchymal located cells in one sample. Oval cells were embedded in fibrous septa or surrounding it. Parenchymal positive ductular cells were found between hepatocytes and also found embedded in centrilobular fibrotic tissue. Hepatocytes also showed a very light CK7 staining in chronic hepatitis samples.

Table 2. Antibody specifications and processing characteristics

| Antibody | Type | Clone | Company | Antigen Retrieval | Dilution |
|-----------|------------|-------------|--|---|--------------|
| CK7 | Monoclonal | OV-TL 12/30 | Thermo Fisher Scientific, Fremont, CA, USA | Citrate buffer (pH 6.0) 2 × 5 min 800 Watt/ 5 min 600 Watt | Ready to use |
| CK19 | Monoclonal | A53 B/A2.26 | Thermo Fisher Scientific | Citrate buffer (pH 6.0) 2 × 5 min 800 Watt/5 min 600 Watt | Ready to use |
| PCNA | Monoclonal | PC10 | Thermo Fisher Scientific | Citrate buffer (pH 6.0) 2 × 5 min 800 Watt/5 min 600 Watt | 1:200 |
| Ki67 | Monoclonal | SP6 | Thermo Fisher Scientific | Citrate buffer (pH 6.0) 2 × 5 min 800 Watt/5 min 600 Watt | 1:300 |
| Hep Par-1 | Monoclonal | OCH1E5 | Sigma-Aldrich, St. Louis, MO, USA | EDTA antigen retrieval (pH 8.0) | 1:150 |

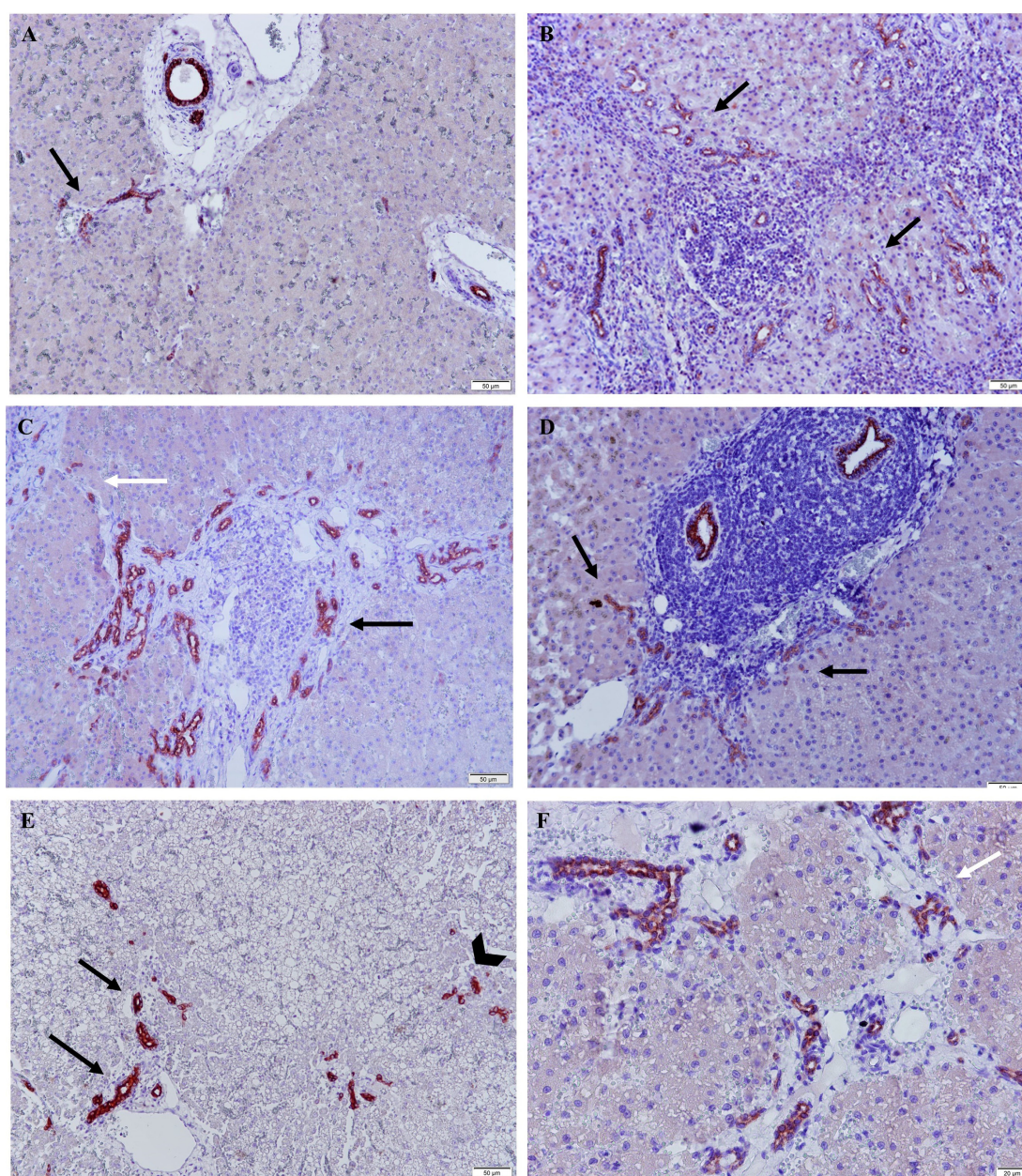


Fig. 1. Cytokeratin (CK) 7 staining in different feline specimens, Cat. A: Mild CK7 positive portal ductular reaction (arrow), passive congestion. B: Prominent CK7 portal and periportal ductular reaction (arrows), fulminant hepatitis. C: Portal, periportal (black arrow) and septal (white arrow) CK7 positive progenitor cells, metastatic lymphoma. D: Portal and periportal ductular reaction surrounding the periductular lymphocytic infiltration (arrows), lymphocytic cholangiohepatitis. E: Portal, periportal (arrows) and parenchymal (arrowhead) CK7 positive ductular reaction, hepatocellular lipidosis. F: CK7 positive ductular reaction embedded in the septal fibrotic structures (white arrow), congenital cystic disease. Bars= 50 µm (A–E), 20 µm (F).

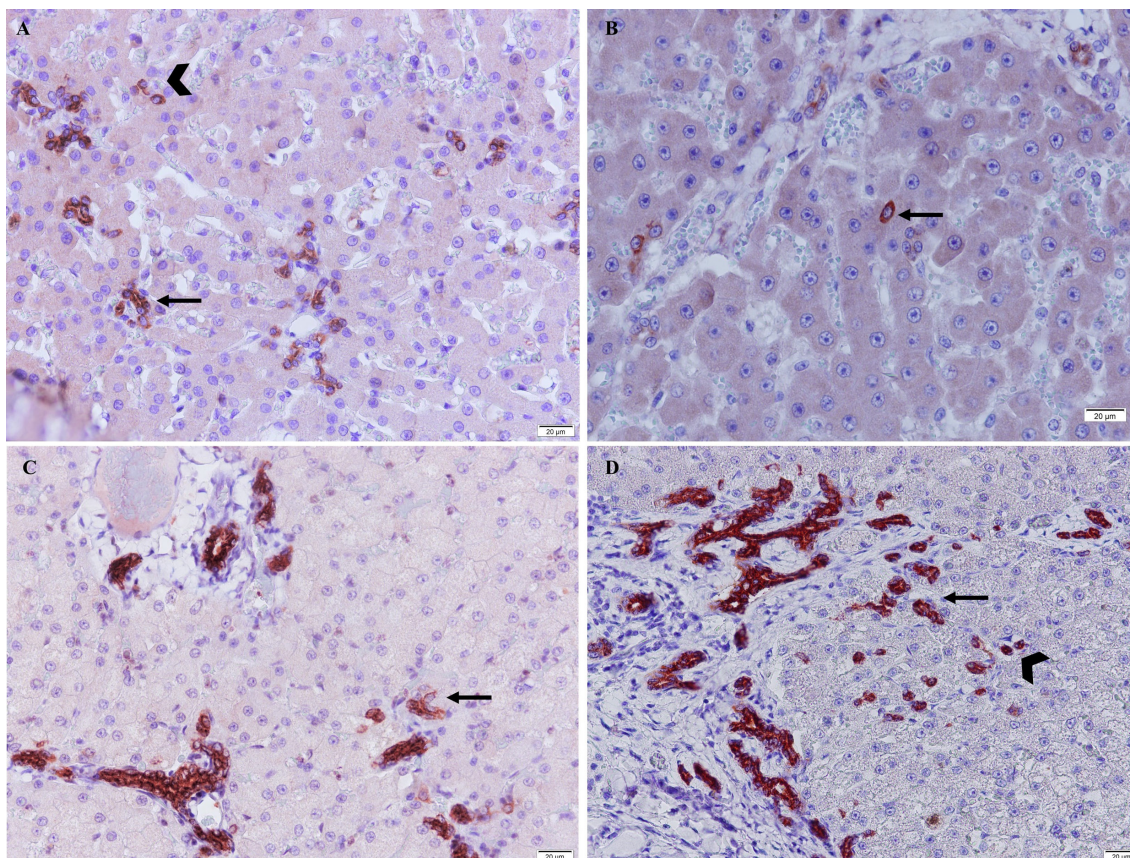


Fig. 2. The different morphological components of ductular reaction, Cat. A: Clusters (arrow) and solitary single oval shaped cells (arrowhead) with strong cytoplasmic staining located in the parenchyma. B: Intermediate cell (arrow) or hepatocyte resembling cell featuring membranous CK7 staining. C: Clusters of progenitor cells radiating into the parenchyma (arrow). D: Small ductules without lumen (arrow) and solitary progenitor cells (arrowhead) mainly periportal.

In two of the metastatic lymphoma cases (cases 19 and 21), metastatic cells showed wide portal infiltration areas that caused severe necrosis and fibrosis in the surrounding parenchyma. Ductular reaction was prominently present and ‘case 19’ presented the most intensive ductular reaction in this study. Increased CK7 positive cells were located in the portal tracts, in the fibrotic septa surrounding tumoral areas, at the interface between the portal tracts and the hepatic parenchyma, and in the periportal regions and they radiated deeper into the parenchyma (Fig. 1C). In the third case of feline metastatic lymphoma (case 20), metastatic tumor cells showed diffuse sinusoidal infiltration of the liver and caused mild degeneration and necrosis in the parenchyma. This case showed mildly increased ductular reaction mainly located in the portal and periportal areas.

Feline liver with hepatocellular adenoma showed negative staining for CK7 in the tumor area and mild positive staining highlighting ductular reaction in the adjacent parenchyma especially located portally and periportal. It should be pointed out that in addition to hepatocellular adenoma, lymphocytic cholangitis was diagnosed in this case too.

Lymphocytic cholangitis/cholangiohepatitis cases showed a mild to moderate CK7 positive ductular reaction localized in portal and periportal areas, surrounding the periductular lymphocytic infiltration (Fig. 1D), with mild parenchymal involvement only in two cases.

Cases diagnosed with hepatocellular lipodosis demonstrated a mild to moderate increase in portal/periportal CK7 positive cells with positive cells located in the parenchyma being observed in 5 cases (Fig. 1E). Parenchymal CK7 positive cells consisted of clusters of strongly positive 4–5 oval cells and small diameter ductules without lumen (Fig. 2). Hepatocyte resembling cells (intermediate cells) with less intensive CK7 positivity were also scattered in the parenchyma. It was noted that the cases showing parenchymal ductular reaction had the highest scores of CK7 positive cells among hepatocellular lipodosis cases and demonstrated a diffuse pattern of fat accumulation. Increased positivity of CK7 positive cells was not found to be correlated with the severity of hepatocellular lipodosis.

Congenital cystic disease of the liver showed prominent increase of CK7 positively stained ductular profiles in the portal tracts, moderate increase of CK7 positive periportal and septal ductular structures, and mild parenchymal involvement. Small caliber bile ducts with or without lumen, clusters, and single oval cells were seen in the portal tracts, the portal bridging fibrous septa, the interface between the portal tracts and the hepatic parenchyma, and the cyst wall and were also found to be embedded in the fibrous tissue surrounding the central veins (Fig. 1F).

PCNA antibody showed nuclear positive staining of some hepatocytes, biliary cells, oval cells, and mesenchymal cells in normal

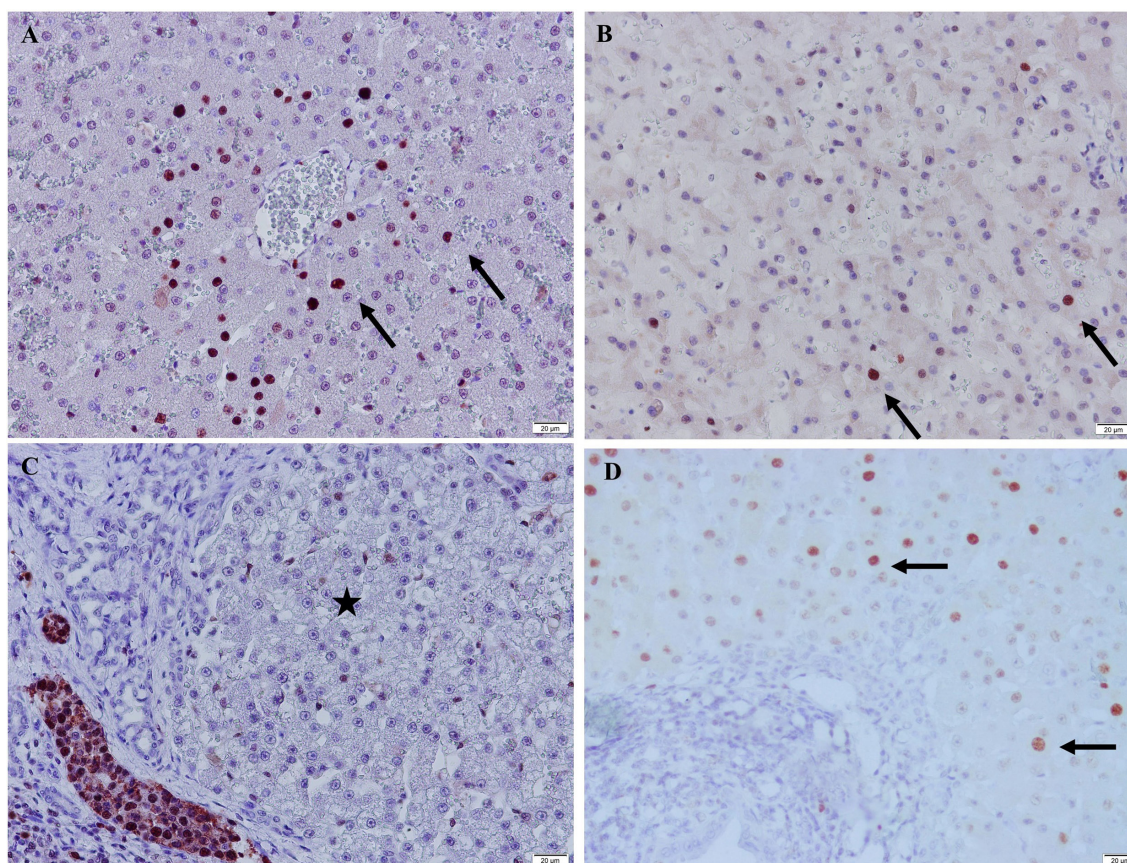


Fig. 3. Proliferating cell nuclear antigen (PCNA) staining in different feline specimens, Cat. A: Prominent PCNA staining of centrilobular hepatocytes (arrows), passive congestion. B: Low hepatocellular PCNA positivity (arrows), fulminant hepatitis. C: Absence of PCNA positive hepatocytes in the adjacent parenchyma (star) with prominent positivity of metastatic lymphoid tumor cells, metastatic lymphoma. D: Periportal hepatocellular PCNA positivity (arrows), lymphocytic cholangiohepatitis.

liver tissue, and it showed nuclear positive staining of some inflammatory cells in affected tissue. Only mitotic activity of the hepatocytes was evaluated. Normal liver showed 1 to 2 positive hepatocytes (<1%) on HPF. Results of PCNA staining in different types of liver diseases are shown in [Table 1](#).

In acute hepatic disease such as hepatic displacement and acute passive congestion, PCNA staining score changed according to the disease severity from low to prominent. While PCNA displayed diffuse hepatocellular staining in hepatic displacement cases, it has a centrilobular staining in passive congestion cases ([Fig. 3A](#)).

PCNA positive hepatocyte proliferation scores in the majority of acute and subacute hepatitis cases (5 out of 6 cases) altered between being moderate to being prominent and they increased with necrotic activity. Besides, the density of PCNA positive cells was especially localized around necrotic areas. For example, in the cases with centrilobular necrosis, PCNA positive hepatocytes were concentrated in midzonal and periportal areas.

Hepatocyte proliferation was low in fulminant hepatitis ([Fig. 3B](#)) and chronic hepatitis cases. No PCNA positive cells were located either in or around necrotic areas. PCNA positivity was conspicuous in metastatic tumor cells of all metastatic lymphoma cases ([Fig. 3C](#)). In contrast, low PCNA positivity of hepatocyte was observed in the adjacent parenchyma ([Fig. 2C](#)).

In hepatocellular adenoma, intense positivity for PCNA was observed in the tumor area and the adjacent parenchyma. Positive hepatocytes were diffusely distributed in the tumor area and were mostly present in the periportal zones of the adjacent parenchyma.

The majority of lymphocytic cholangitis/cholangiohepatitis cases showed low PCNA positivity of hepatocytes, especially periportally localized ([Fig. 3D](#)). Four out of nine hepatocellular lipidosis cases showed mildly positive hepatocyte replication, while the rest showed moderate replication. Congenital cystic disease of the liver showed low positive staining of the hepatocytes.

Like CK7, CK19 stained the cytoplasm of bile duct epithelial cells in normal liver but less intensively ([Supplementary Fig. 1](#)). Progenitor cells and intermediate cells were negative for CK19 antibody.

Immunohistochemical results of Ki67 were similar to those of PCNA in unaffected and diseased feline liver samples; however, non-specific staining was more pronounced ([Supplementary Fig. 2](#)).

Hep Par-1 staining results showed a prominent cytoplasmic reaction in the hepatocytes of the feline normal liver section. Portal biliary ducts showed no positive reaction. Degenerative and necrotic hepatocytes in all the feline diseases displayed a mild to moderate Hep Par-1 cytoplasmic reaction ([Fig. 4A](#)). Single or clusters of oval cells and ductular structures with or without lumen

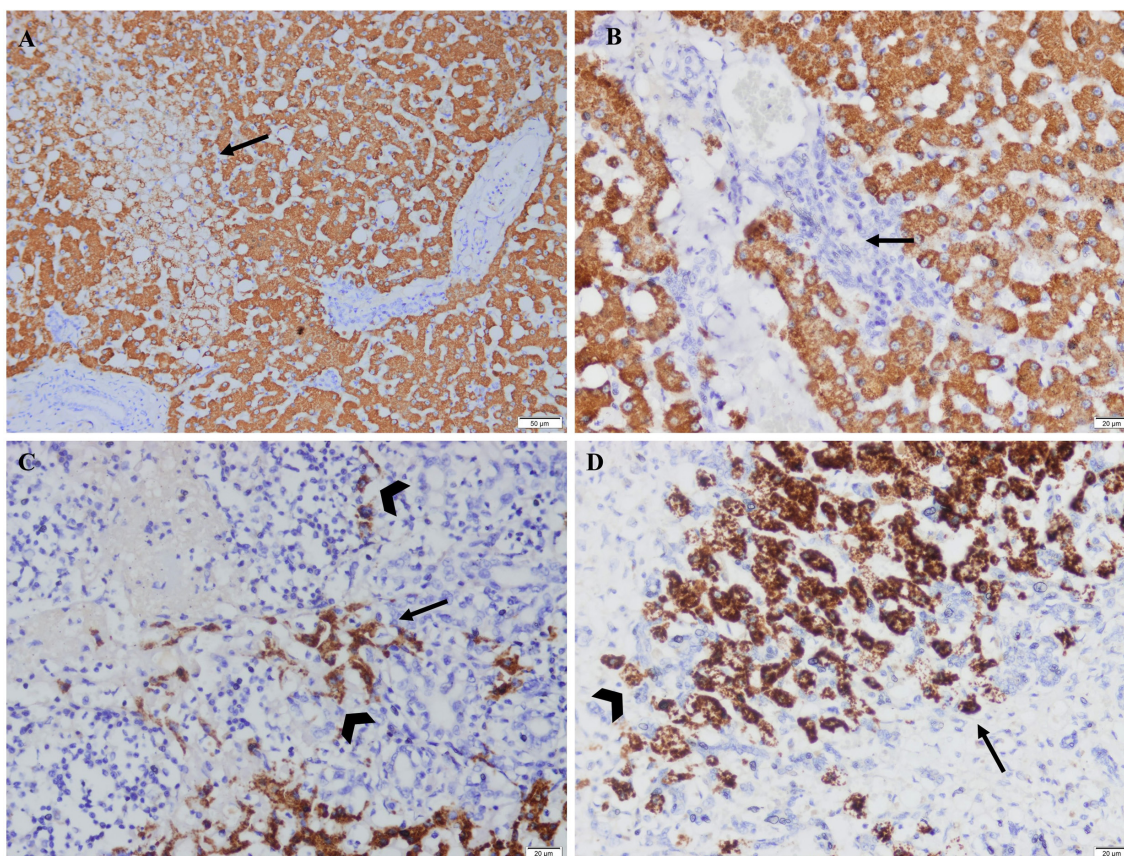


Fig. 4. Human hepatocyte marker 1 (Hep Par-1) staining in various feline specimens, Cat. A: Degenerative hepatocytes shows a mild Hep Par-1 positivity compared to the surrounding mature hepatocytes showing prominent positive staining, hepatocellular lipidosis. B: Negative staining of the majority of the portal ductular structures (arrow), hepatocellular lipidosis. C: Positively staining epithelial cells of the ductular reaction with Hep Par-1. Reactive ductules (arrow), single and clusters of progenitor cells (arrowheads) portally located, fulminant hepatitis. D: Septal ductular reaction strongly reacting with Hep Par-1. Slight granular (arrowhead) and intensive (arrow) cytoplasmic positive progenitor cells, metastatic lymphoma.

forming the ductular reaction were generally negative for Hep Par-1 antibody (Fig. 4B). In cases of fulminant hepatitis, chronic hepatitis, hepatocellular lipidosis and metastatic lymphoma, some ductular structures as well as single or group of cells situated between the ductular structures were positively stained with Hep Par-1 (Fig. 4C, 4D). These cells showed slight granular or intense cytoplasmic positivity and were specially visualized in the portal areas, the septal areas and the interface between the hepatocytes and the ductular structures (Fig. 4D).

DISCUSSION

In the present study, feline progenitor cell compartment's activation was evaluated in comparison with the classical hepatic regeneration pattern by hepatocellular replication in a variety of feline hepatobiliary diseases.

Both PCNA and Ki67 antibodies, used to visualize liver regeneration through hepatocellular mitosis, showed positive staining in feline liver samples; however, PCNA reacted more selectively and specifically. CK7 and CK19, admitted as hepatic stem cell markers in previous studies [2, 5, 14, 19], were used in the present study to reveal liver regeneration through hepatic progenitor cells. CK7 which was proven to react with progenitor cells and intermediate cells is considered a more specific and suitable marker for hepatic stem cells when compared to CK9 and this comes in accordance with the study of Bateman and Hubscher [2].

In line with previous reports [5, 8], in normal feline liver, periportally located individual hepatic progenitor cells showed CK7 positive staining and no intermediate cells were found. In acute/subacute hepatitis and acute hepatic diseases such as hepatic displacement and acute passive congestion, CK7 positive ductular reaction was absent or slightly present whereas PCNA positive replicating hepatocytes were moderately to intensively positive. This may indicate that regeneration of the hepatic parenchyma in acute/subacute hepatitis and acute hepatic diseases (hepatic displacement and acute passive congestion) is predominantly shaped by hepatocellular replication. Ductular reaction was mainly limited to the portal and periportal locations. When present, parenchymal ductular reaction and hepatocellular replication accompanied necrotic activity and were localized around necrotic areas. The centrilobular condensation of PCNA positive cells in acute passive congestion cases is due to centrilobular necrosis caused by hypoxia.

Highest CK7 positive ductular reaction was observed in cases of fulminant hepatitis, chronic hepatitis and metastatic lymphoma

cases (which were considered as chronic cases). Yet, the score of PCNA positive cells was low in all these cases. This indicates the loss of hepatocytes' ability to divide due to severe hepatic damage. Instead, progenitor cells' proliferation increased to meet the replication needs of the liver. Besides portal and periportal ductular reactions, parenchymal ductular reaction involvement, parallel to the severity of hepatocellular necrosis was reported in fulminant hepatitis. Parenchymal and septal ductular reaction, in parallel with necrotic and fibrotic activity, were reported in chronic hepatitis and metastatic lymphoma. Ductular reaction expression in hepatitis cases is similar to what was reported in studies conducted by Ijzer *et al.* [5, 6] and Kruitwagen *et al.* [8]. Interestingly, slight hepatocellular CK7 cytoplasmic staining in fulminant and chronic hepatitis was previously reported by Tan *et al.* [19] in both submassive hepatic necrosis and cirrhosis in human specimens.

Previous studies reported active progenitor cells in feline lymphocytic cholangitis/cholangiohepatitis with portal/periportal predominance [8, 14, 15, 22]. Considered a chronic biliary disease, lymphocytic cholangitis/cholangiohepatitis showed portal and periportal ductular reaction in this study as well. Parenchymal ductular involvement and periportal hepatocellular replication were also reported to be associated with the severity of the disease and its spread toward the hepatic parenchyma.

The results of this study showed reactivity of both hepatocellular replication and ductular reaction in terms of regeneration in feline hepatocellular lipidosis. Although regeneration through hepatocellular division was more active than ductular reaction, the formation of a pronounced ductular reaction in these cases showed that hepatocellular lipidosis can cause serious hepatocellular injury in feline liver. According to the studies conducted by Roskams *et al.* [17] and Yang *et al.* [23], in human and rodent non-alcoholic fatty liver disease models, hepatocellular lipidosis was capable of inhibiting the replication of hepatocytes and triggering the activation of progenitor cells. In a study done by Valtolina *et al.* [21], all feline hepatocellular lipidosis demonstrated ductular reaction with portal, periportal and parenchymal localization but no common pattern of ductular activation could be established. In this study, eight out of nine cases showed ductular reaction with portal, periportal and/or parenchymal localization but the degree of these reactions was not related to the severity of the disease. In this regard, Valtolina *et al.* [21] suggested that this may be due to the variety of underlying diseases that lead to hepatocellular lipidosis.

In congenital cystic disease, low hepatocellular expression of PCNA was reported while pronounced CK7 positive ductular reaction was revealed. Ductular reaction localized mainly in the portal tracts and embedded in fibrotic matrix throughout the parenchyma is considered by Pillai *et al.* [16] a marker of canine ductal plate malformation. Positive ductular reaction displayed in congenital cystic disease is differentiated from the one formed in acquired diseases like acute hepatitis through the absence of accompanying lesions such as necroinflammatory changes. Additionally, the absence of necroinflammatory changes resulting from hepatic injury is thought to be the reason behind the low hepatocellular replication rates in our case of congenital cystic disease.

Hep Par-1 positively stained viable and necrotic hepatocytes with a less intensive coloration for necrotic hepatocytes. While ductular structures generally showed a negative staining for Hep Par-1, they contained positive staining cells in diseased liver sections of a notable ductular reaction such as fulminant hepatitis, chronic hepatitis, hepatocellular lipidosis and metastatic lymphoma. The negative staining of ductular structures is due to their biliary or undifferentiated phenotype and the positive staining of a part of these structures is thought to be due to their acquisition of mature hepatocyte characteristics. Cells showing a slight granular Hep Par-1 staining are considered intermediate hepatobiliary cells. This proves the involvement of these cells in the hepatocellular repopulation of the liver.

In this study, ductular reaction located in the portal, periportal, parenchymal and/or septal sites of hepatic sections with different liver diseases consisted mainly of small ductules with or without distinct lumen along with either single or clusters of progenitor cells. In addition, intermediate cells acquiring hepatocytic phenotype featured a less intense CK7 staining. These cells were not present in congenital cystic disease, hepatic displacement, and acute congestion cases.

In conclusion, this study showed the variation of hepatocellular regeneration patterns in different feline hepatic diseases. Even though in acute events regeneration was predominantly shaped by the division of hepatocytes, in chronic events and in severe acute events (such as fulminant hepatitis) hepatocytes lost their ability to divide and progenitor cells proliferated intensively. Regarding the location, progenitor cells showed portal and periportal predominance of various degrees in most of the cases. Parenchymal ductular reaction was detected in fulminant hepatitis, chronic hepatitis, hepatocellular lipidosis and metastatic lymphoma. Septal ductular reaction was detected only in chronic hepatitis and metastatic lymphoma. In lymphocytic cholangitis/cholangiohepatitis and hepatocellular lipidosis, both ductular reaction and hepatocellular replication were active. Congenital cystic disease showed prominent ductular reaction inside fibrotic structures which is not related to the parenchymal damage but presents a typical feature of the congenital disease.

This study adds to a growing corpus of research showing the importance of progenitor cells in feline hepatic regeneration. Defining a specific pattern of progenitor cell activation in each disease group contributes to the promotion of therapeutic use of progenitor cells in the treatment of some feline hepatic diseases.

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

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