Thesis for the degree of
Master of Veterinary Medicine

Immune-Mediated Lymphocytic Cholangiohepatitis in a Miniature Poodle Dog : Clinical Presentation, Diagnosis and Management

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has been approved by the dissertation committee.

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Abstract

Immune-Mediated Lymphocytic Cholangiohepatitis in a Miniature Poodle Dog : Clinical Presentation, Diagnosis, and Management

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A 2-year-old castrated male miniature poodle was presented with 11 days history of anorexia, weight loss, and icterus. Since the patient deteriorated with fluid therapy and liver aids for 8 days at the local hospital, refered to the hospital for intensive treatment. Serum chemistry revealed that severe hyperbilirubinemia with elevated liver panels. Abdominal ultrasonography revealed remarkable gall bladder wall thickening with no clear evidence of gall bladder lumen dilation or duct obstruction. There was no response to supportive treatment for cholecystitis, therefore cholecystectomy was perfomed. Based on the histopathological results of the gallbladder and liver, lymphocytic cholangioheatitis (LC) which have been rarely reported in dogs was diagnosed. one year postoperatively, the patient visited the hospital

with suspected recurrence. Immunosuppressive treatment was immediately attempted by tentative diagnosis for LC. After taking medicine, the patient's urine, general conditions and appetite were normalized. Since then, the patient has been tapering 20% of immunosuppressants every two weeks, and it is currently maintained well without recurrence of the disease. To the best of our knowledge, this is the first reported case of canine lymphocytic cholangiohepatitis that was achieved remission through surgery and application of immunosuppressants.

Key words: cholangitis, immunosuppressants, dog, hyperbilirubinemia, lymphocytic cholangiohepatitis

Contents

I. Introduction

Cholangitis is inflammation confined to the portal region of the liver with infiltration into the bile duct epitherlium or within the duct lumen. While cholangitis typically refers to the inflammation limited to the bile ducts, cholangiohepatitis extends the inflammation to include the surrounding liver parenchyma. In cholangiohepatitis, the inflammation extends beyond the bile ducts and encompasses the periportal region, indicating a more extensive and severe form of the condition compared to cholangitis alone. Symptoms of cholangiohepatitis resemble those of cholangitis but are typically more severe. The histopathological examination reveals inflammatory cell infiltrates, including lymphocytes, neutrophils, and macrophages, in the bile ducts and liver tissue, accompanied by tissue damage and associated changes. Treatment for cholangiohepatitis follows a similar approach to that of cholangitis. Cholangitis can be categorized into four groups as per the guidelines established by the World Small Animal Veterinary Association (WSAVA) in 2006: neutrophilic cholangitis, lymphocytic cholangitis, destructive cholangitis, and chronic cholangitis which is commonly associated with liver fluke infestation [4]. According to a retrospective report that examined 54 dogs with cholangitis, classified based on the WSAVA guidelines, the majority of dogs were classified with neutrophilic cholangitis, while only one dog was histologically classified as having lymphocytic cholangitis [5], and it is the only report of lymphocytic cholangitis in a dog by now. To the best of our knowledge, there is no further information on dogs with lymphocytic cholangitis, therefore, we could not determine the treatment follow-up.

Feline LC is relatively common [2]. In one study of feline liver biopsy samples from the UK, LC was present in 6.8% of samples [1]. For treatment, the patient commonly treated with immunosuppressive of prednisolone or ursodeoxycholic

acid.

Herein, we present a case of 2-year-old castrated male miniature poodle dog, which was diagnosed with LC that was achieved remission through surgery and application of immunosuppressants.

II. Materials and Methods

Patient

A 2-year-old castrated male Miniature Poodle weighing 4.9 kg presented with 11 days history of anorexia, weight loss, and icterus. As the patient's condition continued to deteriorate despite receiving fluid therapy and liver aids for a duration of 8 days at the local hospital, the patient was refered to the VIP Animal Medical Center (Seongbuk branch, Seoul, Korea) for management.

Laboratory examination

Laboratory evaluations included a complete blood count (CBC) (Procyte Dx Hematology analyzer; IDEXX, USA), assessment of serum chemistry including alanine aminotransferase (ALT, RI, 10 - 125 U/L), alkaline phosphatase (ALP, RI, 23 - 212 U/L), aspartate aminotransferase (AST, RI, 0 - 50 IU/I), total bilirubin (t.bil, RI, 0.0 - 0.9 mg/dL), gamma glutamyl transpeptidase (GGT, RI, 0 - 10 U/L), and triglyceride (TG, RI, 10 - 100 mg/dL), urine protein creatinine ratio (UPC, RI, 0.0 - 0.5) and total tyroxine (tT4), (Catalyst One Chemistry analyzer; IDEXX, USA), canine c-reative protein (CRP) and canine pancreas-specific lipase (cPL) (Vcheck V200, BIONOTE, USA).

Imagimg examination

The abdominal and thoracic radiographs were obtained using digital radiographic equipment (Listem, Seoul, Korea). Abdominal were evaluated by ultrasonography (Philips, Bothell, WA, USA).

Pathologic examinations

Tissue samples were stained with hematoxylin and eosin (H&E) for histopathologic analysis at the laboratory of Veterinary Pathology in ANTECH (Asia Veterinary Diagnostics).

III. Results

There was no history of foreign body ingestion or the ingestion of toxic materials. During the physical examination, the patient's body condition score was determined to be 3/9. Vital signs such as pulse rate, systemic blood pressure, and respiratory rate were within the normal range. The mucous membranes, skin, and sclera exhibited a yellowish discoloration.

There were no specific findings on the CBC. Serum chemistry analysis showed increased in ALT (347 U/L), ALP (882 U/L), AST (511 IU/I), total bilirubin (16.7 mg/dL), GGT (45 U/L), and TG (121 mg/dL); other results, including ammonia, were within the normal range. CRP (31.8 mg/dL) and cPL (418 ng/mL) were high. All electrolyte levels and tT4 (1.6 ng/ml) were in the normal range. Urinalysis revealed bilrubinuria (3+), and proteinuria (2+). UPC was in the normal range.

Thoracic and abdominal radiography revealed non-specific findings. Abdominal ultrasonographic examinations revealed gall bladder wall thickening of approximately 2.3 mm. No significant findings were observed in terms of gall bladder lumen dilation, gall bladder stones, or abnormalities in the common bile duct (Fig. 1). Hypoechogenicity of the pancreas was noted also. Considering the ultrasonographic findings, acalculous infectious cholecystitis and acute chronic pancreatitis were suspected. We were unable to perform further diagnostic procedures including percutaneous ultrasound-guided cholecystocentesis for bile collection due to the owner's refusal.

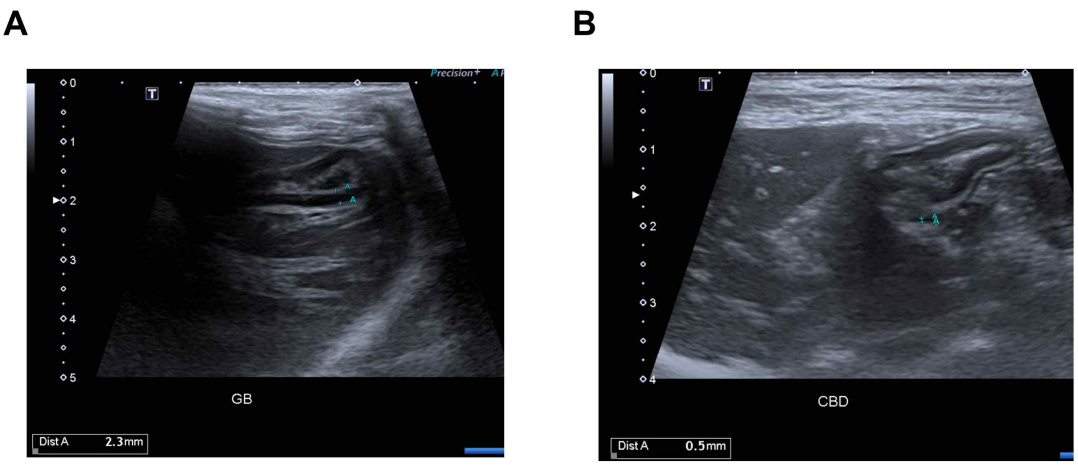


Figure 1. Sagittal ultrasound image of the gall bladder (GB; A), and common bile duct (CBD; B).

During hospitalization, the patient received supportive treatment for infectious cholecystitis with fluid therapy, antibiotics including amoxicillin-clavulanic acid (12.5 mg/kg, per oral, q12h; Lactamox Tab., Aprogen Pharm, Seoungnam, Korea), metronidazole (15 mg/kg, intravenous injection, q12h, Metrynal Inj., Daihan Pharm Co, Seoul, Korea), enrofloxacin (10 mg/kg, per oral, q24h; Baytril Tab., Bayer Animal Health, Suwon, Korea). In addition, liver aids including silymarin (10 mg/kg, per oral, q12h; Legalon Cap., Bukwang Pharm Co, Seoul, Korea), and ursodeoxycholic acid (10 mg/kg, per oral, q24h; Urusa Tab., Daewoong Pharmaceutical Co, Seoul, Korea), and anti-inflammatory drugs (prednisolone 1 mg/kg, per oral, q24h; Solondo Tab., Yuhan, Seoul, Korea) were prescribed.

However, after five days of hospitalization, serum total bilirubin concentration was continuously increased by 31.1 mg/dL, and hematocrit (HCT; RI, 37.5-57.5%) was decreased reached down to 20% without reticulocytosis. The patient's lethargy worsened, and gastrointestinal symptoms, including severe vomiting and diarrhea, became increasingly severe. Among the causes of anemia, infection was excluded as negative on vector-borne anemia polymerase chain reaction. Chronic inflammatory conditions with gastrointestinal bleeding considered the cause of the non-regenerative anemia. Therefore, it was considered that the dog was not ameliorated by supportive treatment, and we decided to perform surgical intervention. Before surgery, the patient received two times of blood transfusion with packed red blood cells. Cholecystectomy with gall bladder and liver biopsy, and bile culture were performed subsequently. The liver was generally discolored, appearing dark brown (Fig. 2A), and the gall bladder was atrophied (Fig. 2B). Histopathological examination of removed gall bladder and biopsy samples of the liver were performed.

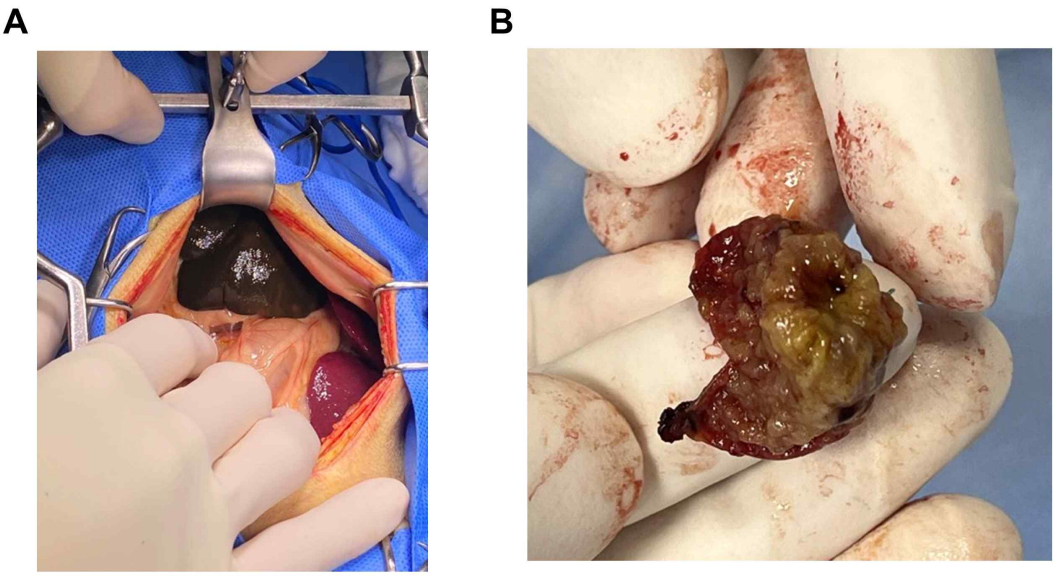
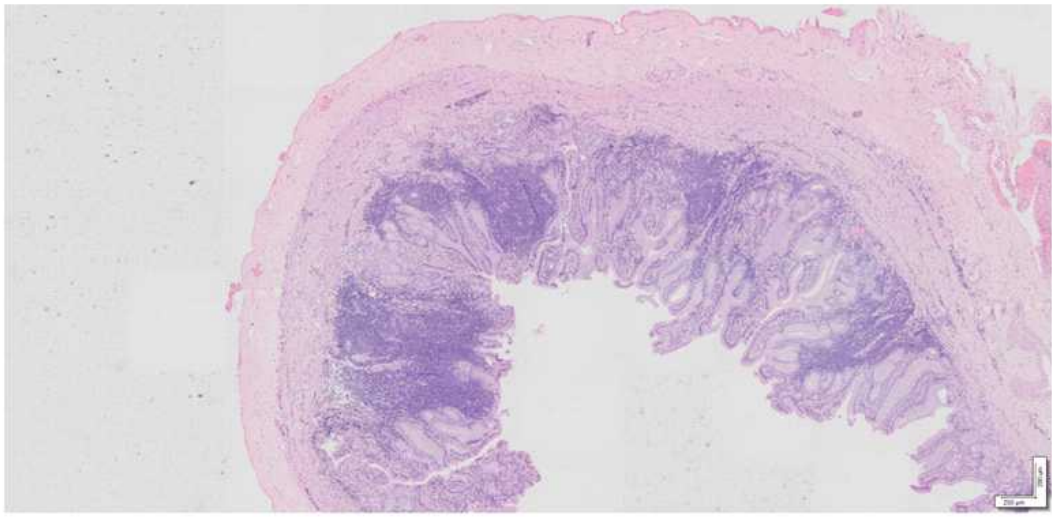


Figure 2. Intraoperative image of the liver (A) and gall bladder (B).

The samples were routinely processed for histopathology and stained with hematoxylin and eosin (H&E). The microscopic examination of the gallbladder revealed a moderate to marked expansion of the lamina propria, caused by an increased number of lymphocytes. The lymphocytic inflammatory component extends into the submucosa. Fewer plasma cells and even fewer neutrophils are admixed with these cells. In some areas, the lymphocytic cells extend into the overlying mucosal epithelium. The histopathological examination of the gallbladder revealed severe lymphocytic cholecystitis (Fig. 3A, 3B). In the liver, there are multifocally expanding portal regions that show a pleomorphic population of lymphocytes previously described in the gallbladder. These cells are accompanied by fewer plasma cells, small lymphocytes, and neutrophils. In some areas, these cells extend into the adjacent parenchyma. The liver showed moderate lymphocytic cholangitis with hepatocellular vacuolar changes and evidence of cholestasis (Fig. 4A, 4B). Collectively, both the gallbladder and the liver contain similar inflammatory cells. Also, microbial culture of the bile showed no growth, suggesting that there was no infection. Based on these histopathologic results, lymphocytic cholangiohepatitis (LC) was diagnosed.

A



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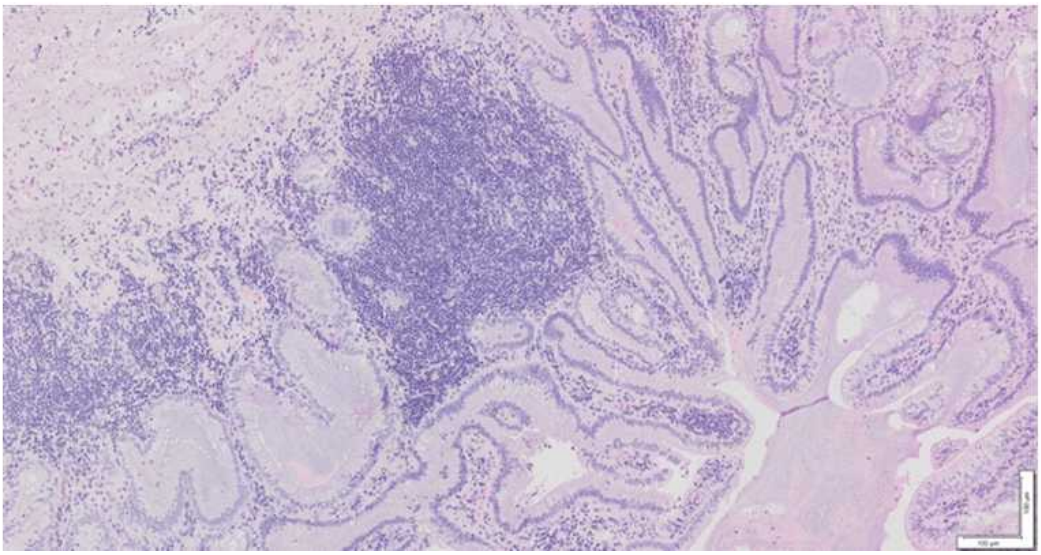
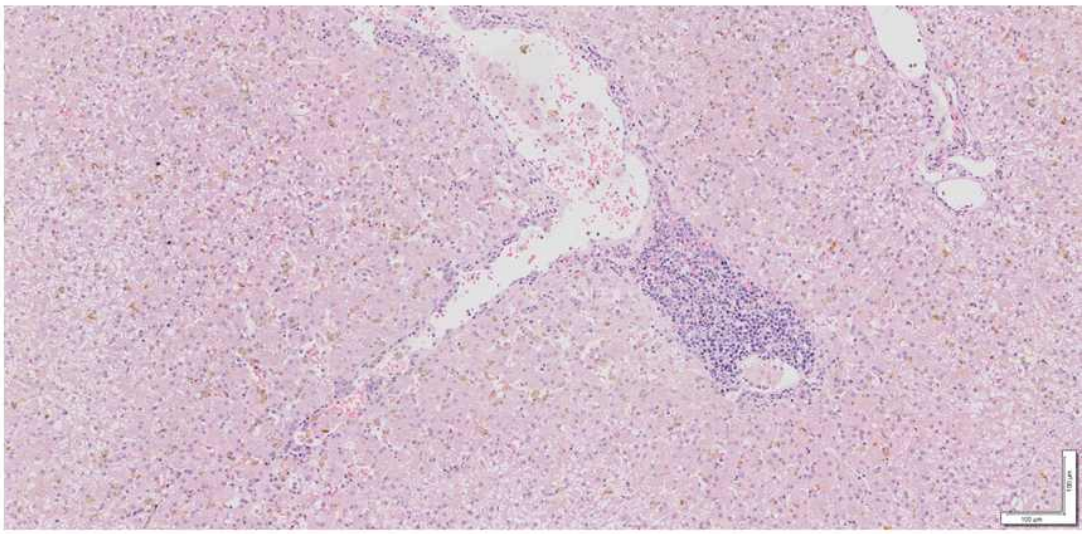


Figure 3. Histopathology with hematoxylin and eosin (H&E) staining of the gall bladder (A), (B). Bars = 100 μm.

A



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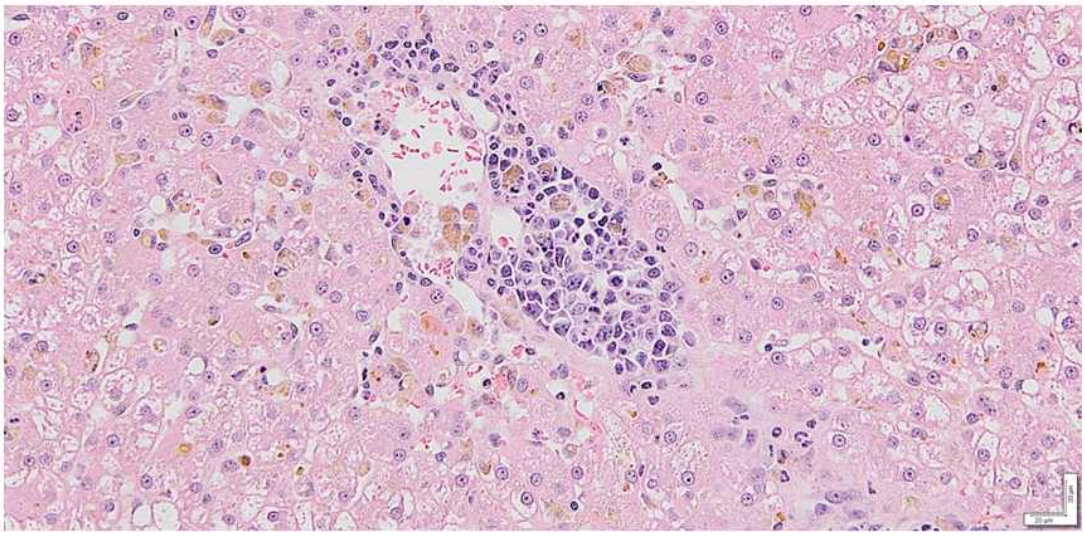

Figure 4. Histopathology with hematoxylin and eosin (H&E) staining of the liver (A), (B). Bars = 100 μm.

After surgery, the liver panel including the total bilirubin level decreased immediately. Although red blood cells regeneration became active, HCT decreased due to blood loss during surgery, and the patient received another blood transfusion with packed red blood cells. Following 11 more days of hospitalization, the patient's appetite and general condition improved, and he was discharged with liver aid and antibiotics.

The total bilirubin level normalized one and a half months postoperatively, and the other liver levels normalized five months postoperatively. Then the patient was retested every month, and well maintained with general conditions, blood tests and ultrasounds. However, one year postoperatively, the patient visited the hospital due to a sudden lethargy and brown urination. Serum chemistry analysis showed an overall increase in total bilirubin (6.9 mg/dL) and ALP (411 U/L), ALT (638 U/L), GGT (15 U/L), AST (409 IU/I). Urinalysis revealed bilirubinemia (3+) and bilirubin (3+). Overall bile duct wall thickening was observed in the patient's ultrasound findings, total bilirubin and liver level increased in blood tests, and LC was confirmed in immunohistochemistry histopathology a year ago, so it was tentatively diagnosed with LC. Although the exclusion of infections in bile did not progress due to the guardian's refusal, immunosuppressive treatment for LC was attempted by tentative diagnosis. Immunosuppressive medications were immediately attempted for treatment (Fig. 5).

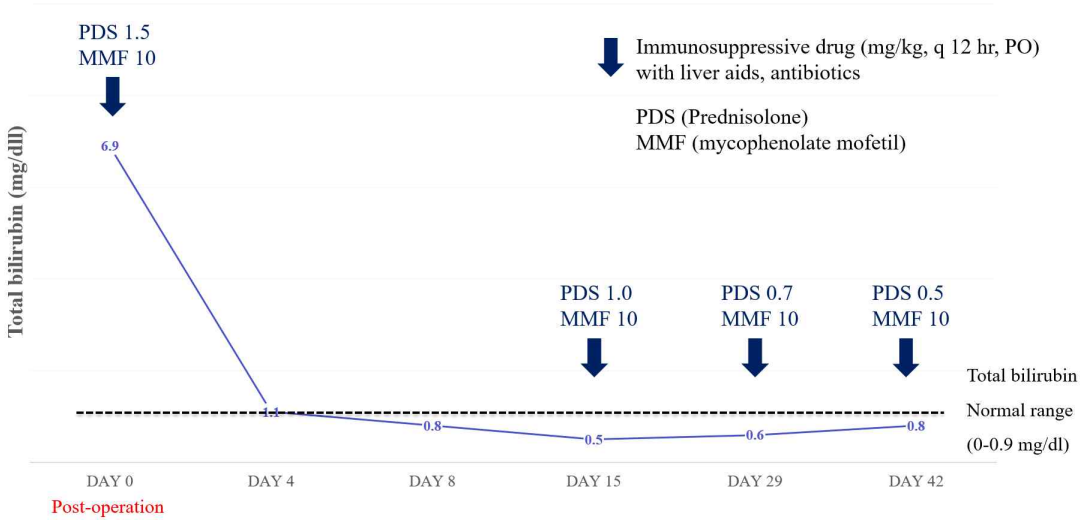

Figure 5. Graph showing changes in total bilirubin levels following the application of immunosuppressants.

The patient administered immunosuppressive drugs with prednisolone (1.5 mg/kg, per oral, q12h) and mycophenolate mofetil (10 mg/kg, per oral, q12h; My-rept Cap., Chong Kun Dang, Seoul, Korea), antibiotics including amoxicillin-clavulanic acid (12.5 mg/kg, per oral, q12h; Lactamox Tab., Aprogen Pharm, Seoungnam, Korea), metronidazole (15 mg/kg, intravenous injection, q12h, Metrynal Inj., Daihan Pharm Co, Seoul, Korea), famotine (0.5 mg/kg, per oral, q12h), and also liver aids including silymarin (10 mg/kg, per oral, q12h; Legalon Cap., Bukwang Pharm Co, Seoul, Korea), and ursodeoxycholic acid (10 mg/kg, per oral, q24h; Urusa Tab., Daewoong Pharmaceutical Co, Seoul, Korea), vitamin-E (tocopherol, per oral, q24h). Eight days after taking medicine, the patient's urine, general conditions and appetite were normalized. Blood test showed that total bilirubin levels were also normalized and were good except for a slight increase in liver levels and liver steroid hepatopathy suspected in ultrasound. Since then, the patient has been tapering 20% of immunosuppressants every two weeks, it is currently has been maintained well without recurrence of the disease.

IV. Discussion

Since there are no reports of treatment for LC in dogs, and because of its extremely rare occurrence, no treatment guideline for LC in dogs is available. In this case, the dog was prescribed prednisolone at 1 mg/kg q 24 hr for 1 week as an anti-inflammatory treatment before surgery. The application of the anti-inflammatory dose of prednisolone was ineffective. The dog had been normalized after cholecystectomy, but 1 year later recurred. Immediately the dog was prescribed with immunosuppressants including immune-suppressive dosage of prednisolone at 1.5 mg/kg q 12 hr and mycophenolate mofetil 10 mg/kg q 12 hr, then the dog improved rapidly.

In contrast, the prevalence of lymphocytic cholangitis is high in cats [2]. In one study of 1,452 feline liver biopsy samples from the UK, NC was diagnosed in 20.5% and lymphocytic cholangitis was present in 6.8% of samples [1]. In the treatment of feline lymphocytic cholangitis, immunosuppressive doses of prednisolone or ursodeoxycholic acid (UDCA) are commonly prescribed. Clinical evaluation conducted on cats with lymphocytic cholangitis revealed that the use of prednisolone resulted in a statistically significant extension of survival time compared to the administration of UDCA. Prednisolone is started at 2 - 4 mg/kg PO q 24 hr and slowly tapered over 6 - 8 weeks to 0.5 - 1 mg/kg PO q 24 - 48 hr [9, 10]. Some cats may require other immunosuppressive medications (e.g., chlormabucil and methotrexate) for lymphocytic cholangitis [3].

In humans, there are five distinct patterns in which it can be classified: granulomatous cholangitis, ductopenia, acute cholangitis/pericholangitis, lymphocytic cholangitis, or non-specific cholangitis [6]. Among the 14 cases included in the study and classified as lymphocytic cholangitis, 10 cases were

attributed to immunemediated causes, one case was associated with primary biliary cirrhosis, and one case was with primary sclerosing cholangitis. Two cases classified as lymphocytic cholangitis patterns were due to bile duct obstructions [6]. Primary sclerosing cholangitis (PSC), a type of lymphocytic cholangitis in humans, exhibits histopathological characteristics resembling those of feline lymphocytic cholangitis. These features include inflammation in the portal tracts, proliferation and fibrosis of bile ducts. Alternative options such as immunosuppressive therapy or liver transplantation may be considered, if conventional treatment using liver aids fails to provide effective results.

There is a limitation in this case report. Despite the extremely rare occurrence of gall bladder or bille duct lymphoma [8], immunohistochemistry analysis of the samples might be suggested to evaluate whether neoplastic lymphocytes are infiltrated. However, it could not be performed in the case. Considering that predominance of small lymphocytes in the H&E histopathologic findings, and the response to immunosuppressants, a diagnosis of LC could be made.

V. Clinical relevance

To the best of our knowledge, this is the first case report of lymphocytic cholangiohepatitis in a dog that was achieved remission through surgery and application of immunosuppressants.

VI. References

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국문 초록

미니어처 푸들개의 면역매개성 림프구성 담관간염 : 임상형태, 진단 및 관리

박세영

제주대학교 대학원 수의학과

(지도교수: 송우진)

2살 거세된 수컷 미니어처 푸들이 11일간 지속된 식욕부진, 체중 감소, 그리고 황달을 호소하였다. 지역병원에서 8일간 수액처치와 간보조제 투약하였으나 점차 악화되어 VIP 동물의료센터 성북점으로 의뢰되었다. 혈청화학검사에서 간수치의 상승, 특히 심각한 고빌리루빈혈증이 발견되었다. 복부 초음파 검사에서 담낭의 확장이나 담관 폐색에 대한 명확한 증거 없이 매우 두꺼워진 담낭벽이 관찰되었다. 담낭염에 대한 보존 치료에 대한 반응이 없었기 때문에, 담낭 절제술이 시행되었다. 담낭과 간의 조직병리학적 결과를 바탕으로 개에게서 드물게 보고된 림프구성 담관간염(LC)을 진단했고, 수술 후 환자는 임상증상 없이 매우 잘 지냈다. 환자는 수술 후 1년만에 재발이 의심되어 병원을 다시 내원했다. 림프구성 담관염에 대한 잠정적인 진단에 의해 재발에 대해 면역 억제 치료가 즉시 시도되었다. 약을 복용한 후 환자의 소변, 일반적인 상태, 식욕이 정상화되었다. 이후 환

자는 2주에 한 번씩 면역억제제를 20%씩 감량하였으며, 이후에도 병의 재발 없이 잘 유지되고 있다. 저자가 아는 한, 이 논문은 수술과 면역억제제의 적용을 통해 완화된, 개에서 림프구 담관간염의 첫 번째 증례 보고이다.

Key words: 담관염, 면역억제제, 개, 고빌리루빈혈증, 림프구성 담관염