Case Report

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Successful treatment of acute-on-chronic liver failure and hemolytic anemia with hepato-protective drugs in combination with intravenous ozone without steroids: A case report

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Summary **Both acute-on-chronic liver failure (ACLF) and autoimmune hemolytic anemia (AIHA) are common causes of jaundice. A co-occurrence of ACLF and AIHA is rare in clinical practice. This report describes a male elderly patient who developed persistently increased levels of total bilirubin and ascites after endoscopic retrograde cholangiopancreatography for the successful treatment of common bile duct stones. Eventually, he was diagnosed with ACLF and AIHA according to current diagnostic criteria. The patient was given conventional hepato-protective drugs, human albumin, and diuretics in combination with immune ozone without steroids, and he responded well. The therapeutic role of immune ozone in this case is also discussed. When immune ozone was given, total bilirubin gradually decreased; however, no change in total bilirubin was observed after immune ozone was stopped. Notably, when immune ozone was re-initiated, total bilirubin decreased again.**

Keywords: Acute-on-chronic liver failure, hemolytic anemia, liver cirrhosis, jaundice, immune ozone

1. Introduction

Acute-on-chronic liver failure (ACLF) is potentially life-threatening (*1*). According to the guidelines of the Asia Pacific Association of Liver Diseases (APASL), ACLF is diagnosed if a patient with previously diagnosed or undiagnosed chronic liver disease/cirrhosis has the following symptoms: jaundice (serum total bilirubin (TBIL) > 5 mg/dL [85 umol/L]), coagulopathy (international standardization ratio (INR) > 1.5 or prothrombin activity < 40%), and development of ascites and/or encephalopathy within 4 weeks (*1*). Treatment of ACLF mainly includes comprehensive medical treatment, artificial extracorporeal liver support, and liver transplantation (*2*).

Autoimmune hemolytic anemia (AIHA) is an acquired heterogeneous autoimmune disease characterized by autoantibodies attacking antigens on autologous red blood cells (RBCs), resulting in destruction of RBCs (*3*). AIHA is a relatively rare disorder, with an estimated incidence of 1 to 3 cases in 100,000 persons per year (*4*). AIHA can occur at any age, but its risk increases with age, and particularly so after the age of 40 (*5*). Diagnostic criteria for AIHA include: *i*) anemia defined as a decreased hemoglobin level; *ii*) the presence of erythrocyte autoantibodies; and *iii*) at least one of the following conditions: a percentage

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of reticulocytes > 4% or an absolute value > 120 × 10^9/L, along with a globin level < 100 mg/L, and TBIL ≥ 17.1 umol/L (mainly an increase in indirect bilirubin [IBIL]) (*6*). Treatment of AIHA mainly includes blood transfusions, glucocorticoid therapy, splenectomy, administration of rituximab, and administration of cytotoxic immunosuppressive agents (*6*).

Immune ozone, also known as ozonated autologous blood therapy, has been used for nearly 40 years. Ozone therapy is widely used for cardiovascular, gastrointestinal, genitourinary, central nervous, head and neck, musculoskeletal, subcutaneous tissue, and peripheral vascular diseases (*7*). Ozone may be effective for the management of some vascular diseases (*8*). However, it has not been accepted as a standard therapeutic modality or by orthodox medicine (*9*). Bocci pointed out that ozonated blood can readily maintain the lifespan of RBCs in the circulatory system (*10*).

The current report describes an elderly male patient with ACLF and AIHA who responded well to hepato-protective drugs combined with immune ozone.

2. Case Report

On February 2018, an 81-year-old male presented with abdominal pain and a fever and was treated with traditional Chinese medicine at a local hospital. On March 2018, he developed abdominal pain and a fever again. Abdominal computed tomography (CT) showed abnormal morphology of the pancreas (Figure 1). Magnetic resonance cholangiopancreatography (MRCP) showed choledocholithiasis (Figure 2). He received conservative treatment at a local hospital, and his symptoms abated.

On April 2, 2018, he developed abdominal pain and a fever after diarrhea with a yellowing of the skin and eyes and was admitted to the Emergency Department at this Hospital. Abdominal CT scans showed liver cirrhosis, abnormal morphology of the pancreas, ascites, and choledocholithiasis (Figure 3). Laboratory results indicated that serum TBIL was 79.0 umol/L (reference range: 5.1-22.2 umol/L), direct bilirubin (DBIL) was 60.8 umol/L (reference range: 0-8.6 umol/L), serum amylase (AMY) was 1,840.00 U/L (reference range: 30-110 U/L), and serum lipase (LIPA) was > 6000 U/L (reference range: 23-300 U/L).

On April 4, 2018, the patient underwent endoscopic retrograde cholangiopancreatography (ERCP) in Hepatobiliary Surgery at this Hospital. A stone 0.6 × 0.5 cm in size was removed from the common bile duct. Abdominal pain was greatly alleviated. However, jaundice worsened, and TBIL and DBIL levels continued to increase (Figure 4). In addition, gross ascites developed and gradually worsened. On April 8, 2018, the patient underwent a cholangiography *via* a naso-biliary drainage catheter that suggested the patency of the common bile duct. On April 13, 2018, the patient underwent laboratory tests for hepatitis A, hepatitis E, hepatitis B, hemolysis, immunity-related liver diseases, and tumor markers. Viral hepatitis and immunity-related liver diseases were ruled out. Hemolysis tests indicated that erythrocyte osmotic fragility (primary dissolution) was 0.35% (reference range: 0.40-0.45%), erythrocyte osmotic fragility (completely soluble) was 0.30% (reference range: 0.35-0.40%), and the Coombs test was positive.

On April 16, 2018, the patient was transferred to Gastroenterology at this Hospital. His disease history was reviewed in detail. He suffered from intractable insomnia in 1998 and took alprazolam tablets for more than 10 years. He had no history of hypertension. He had never smoked nor drunk alcohol. Laboratory results indicated that the RBC count was 2.58 × 10^{12}/L (reference range: 4.0-5.5 × 10^{12}/L), hemoglobin (Hb) was 96 g/L (reference range: 110-150 g/L), the percentage of reticulocytes was 4.71% (reference range: 0.5-2.0%), the reticulocyte count was 0.122 × 10^9/L (reference range: 0.015-0.1 × 10^9/L), TBIL

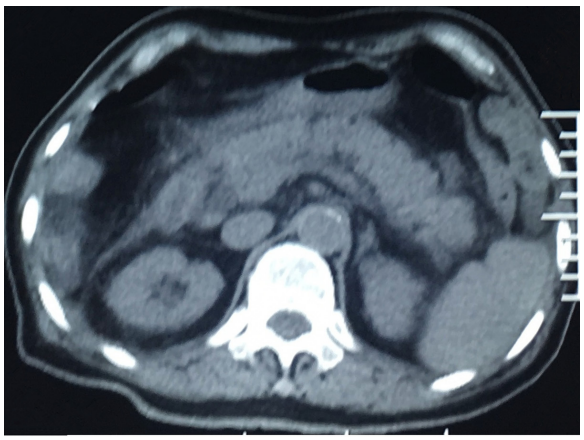

Figure 1. Abdominal CT was performed at a local hospital on March 2018 and showed abnormal morphology of the pancreas.

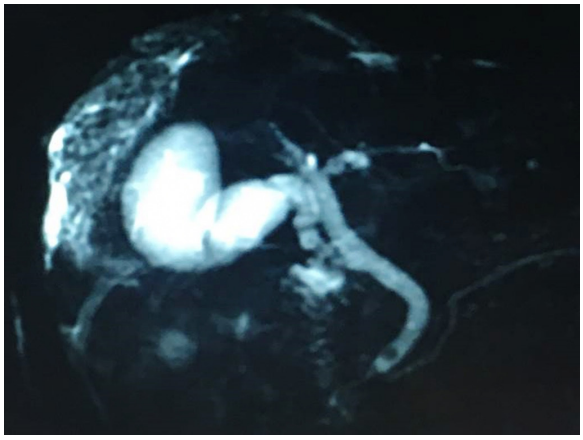

Figure 2. Magnetic resonance cholangiopancreatography was performed at a local hospital on March 2018 and showed an enlarged gallbladder and a stone at the end of the common bile duct.

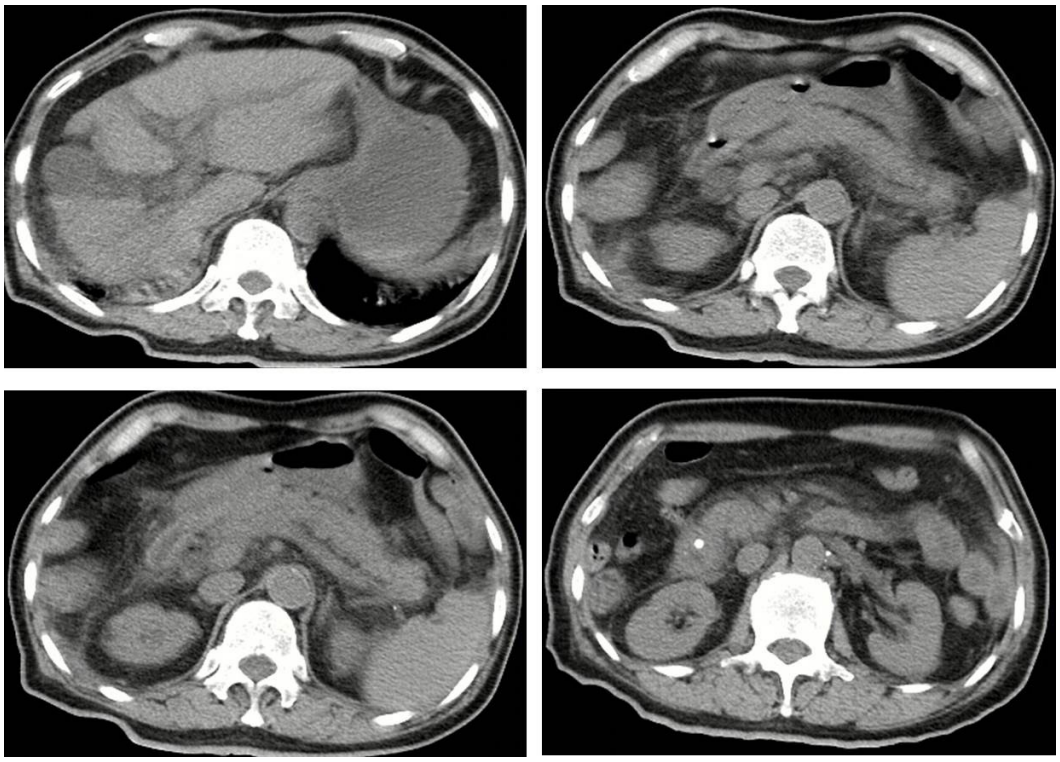

Figure 3. Abdominal CT scans at this Hospital on April 2, 2018 showing liver cirrhosis, abnormal morphology of the pancreas, ascites, and choledocholithiasis.

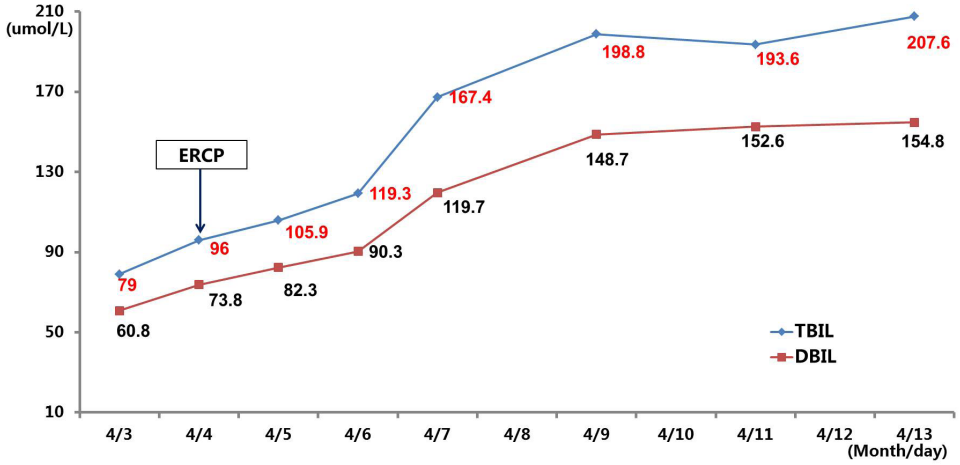

Figure 4. Changes in TBIL and DBIL after ERCP in Hepatobiliary Surgery at this Hospital.

was 186.5 umol/L, DBIL was 140.1 umol/L, alanine amino-transaminase (ALT) was 53.84 U/L (reference range: 9-50 U/L), aspartate amino-transaminase (AST) was 90.5 U/L (reference range: 15-40 U/L), alkaline phosphatase (AKP) was 161.78 U/L (reference range: 45-125 U/L), γ-glutamyl transpeptidase (GGT) was 106.49 U/L (reference range: 10-60 U/L), albumin (ALB) was 25.9 g/L (reference range: 40-55 g/L), prothrombin time (PT) was 21.5 s (reference range: 11.5-14.5 s), INR was 1.8, serum immunoglobulin (IgG) was 24.77 g/L (reference range: 6-16 g/L), and serum immunoglobulin (IgA) was 4.84 g/L (reference range: 0.71-3.35 g/L). He was diagnosed with liver cirrhosis and ACLF. His Child-Pugh score was 13 points. A consultation with a hematologist also suggested a diagnosis of AIHA. Since the patient had a fever and a potential infection, steroids were ruled out. Adenosine methionine, glutathione, isoglycyrrhizinate, folate tablets, vitamin B12, furosemide, spironolactone, and human albumin were given. In addition, intravenous immune ozone was prescribed as an adjuvant therapy.

On April 24, 2018, laboratory results indicated that ALT was 53.67 U/L, AST was 91.09 U/L, GGT was 96.10 U/L, AKP was 154.58 U/L, TBIL was 121.2 umol/L, DBIL was 101.7 umol/L, ALB was 28.2 g/L, PT was 20.0 s, serum AMY was 82.00 U/L, and serum LIPA was 228.0 U/L. At this time, the patient refused intravenous immune ozone due to the potential

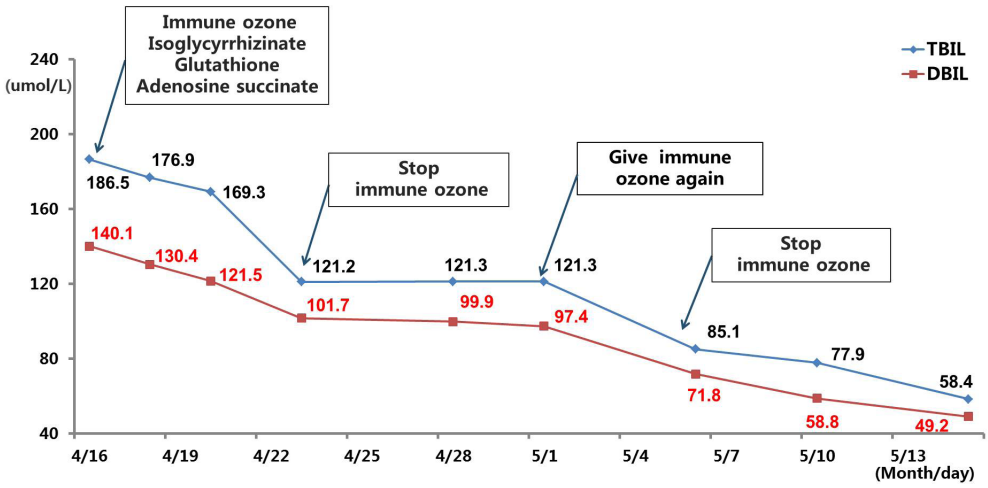

Figure 5. Changes in TBIL and DBIL in Gastroenterology at this Hospital.

invasiveness of the procedure.

On May 1, 2018, laboratory results indicated that ALT was 79.25 U/L, AST was 123.15 U/L, AKP was 154.68 U/L, GGT was 110.65 U/L, TBIL was 121.3 umol/L, DBIL was 97.4 umol/L, ALB was 34.3 g/L, PT was 17.3 s, serum AMY was 107.00 U/L, and serum LIPA was 222.0 U/L. Heeding the advice of his physician, the patient agreed to initiate immune ozone again.

On May 10, 2018, laboratory results indicated that ALT was 64.3 U/L, AST was 94.08 U/L, AKP was 139.03 U/L, GGT was 112.53 U/L, TBIL was 77.9 umol/L, DBIL was 58.8 umol/L, ALB was 34.1 g/L, PT was 16.1 s, serum AMY was 113.00 U/L, and serum LIPA was 244.0 U/L.

On May 16, 2018, laboratory results indicated that ALT was 59.64/L, AST was 92.55 U/L, AKP was 140.23 U/L, GGT was 106.15 U/L, TBIL was 58.4 umol/L, DBIL was 49.2 umol/L, and ALB was 32.5 g/L (Figure 5). Hemolysis tests were performed again. Erythrocyte osmotic fragility (primary dissolution) was 0.45%, erythrocyte osmotic fragility (completely soluble) was 0.40%, and the Coombs test was negative. Ascites was not evident on ultrasound. Thus, the patient was discharged. Oral polyene phosphatidylcholine capsules and silymarin tablets were prescribed.

On May 24, 2018, laboratory tests were performed again. The RBC count was 3.78×10^{12}/L, Hb was 129 g/L, TBIL was 54.2 umol/L, DBIL was 43.5 umol/L, ALT was 54.31 U/L, AST was 72.21 U/L, AKP was 124.73 U/L, GGT was 82.05 U/L, ALB was 35 g/L, PT was 15.9 s, and INR was 1.28.

On June 27, 2018, laboratory tests were performed again. The RBC count was 3.78×10^{12}/L, Hb was 138 g/L, TBIL was 29.8 umol/L, DBIL was 16.6 umol/L, ALT was 48.44 U/L, AST was 55.22 U/L, AKP was 138.35 U/L, GGT was 49.14 U/L, and ALB was 32 g/L. The patient is in satisfactory condition without any complaints.

3. Discussion

An interesting aspect of this case is that the TBIL level successively increased to more than 200 umol/L after a common bile duct obstruction was relieved following ERCP. Thus, the causes of jaundice needed to be examined. First, viral hepatitis, autoimmune hepatitis, and tumor markers were negative. Second, since the patient had a prior history of taking medication, drug-related liver injury was suspected. However, the patient had a RUCAM (*11*) score of 2 points. Thus, drug-induced liver injury was unlikely. Third, since the patient had a high serum IgG antibody titer, IgG4-related cholangitis was suspected. However, this possibility was ruled out based on the diagnostic criteria for IgG4-related cholangitis (*12*) and the IgG4 level. Fourth, the patient's WBC and GR% gradually increased after ERCP, indicating that the patient might have a biliary infection. Thus, the rise in TBIL and DBIL levels after ERCP might have been caused by a persistent biliary infection. Notably, the patient's inflammatory indices returned to normal after the administration of antibiotics, but his TBIL and DBIL levels continued to rise. Thus, the possibility that TBIL and DBIL increased due to biliary tract infection alone was ruled out. Fifth, CT scans performed at this Hospital suggested a diagnosis of liver cirrhosis. However, the etiology of liver cirrhosis in this patient was unclear. In China, the most common etiological factors are viral hepatitis and alcohol consumption. However, viral hepatitis and a history of alcohol consumption were not evident in this patient. Given his medical history, the possible causes of cirrhosis may have included schistosomiasis, chronic use of drugs, and long-term cholestasis. However, a liver biopsy was not performed, so a definite etiology of liver cirrhosis could not be determined. In addition, the patient said that his schistosomiasis had been cured and that he had stopped taking some drugs. In this patient, repeated episodes of cholestasis may have constituted

the true etiology of liver disease. Sixth, according to the diagnostic criteria for ACLF of the APASL, the patient was diagnosed with ACLF. Seventh, the patient had a gradually decreased level of Hb, an elevated percentage of reticulocytes, and a positive Coombs test, so he was also diagnosed with AIHA (*6*).

The treatment strategy in this case should also be examined. Intravenous ademetionine, glutathione, isoglycyrrhizinate, and albumin infusions were effective in treating ACLF. Blood transfusions are a potentially risky treatment for AIHA because the presence of autoantibodies may increase the difficulty of cross-matching and the risk of hemolytic transfusion reactions (*6*). Since the patient had only mild anemia, blood transfusions were not given. In addition, the use of steroids was initially considered in this case. At the time, however, the patient suddenly developed a fever. After balancing the risk and benefits of steroids, the patient and his family members decided to continue following a wait-and-see strategy and they refused steroids.

Another interesting finding is that immune ozone might have alleviated jaundice in this case. Ozone can be dissolved in the aqueous components of plasma, thereby triggering oxidative stress in the body and producing antioxidants, such as superoxide dismutase, glutathione peroxidase, and glutathione S-transferase, catalase, heme oxygenase-1, heat shock protein, and phase II drug-metabolizing enzymes, which are free radical scavengers (*13*). Treatment with ozone is effective in reducing the levels of anti-erythrocyte and anti-leukocyte antibodies and other antibodies (*14*). Notably, after discontinuing the immune ozone, the TBIL level plateaued for nearly a week. In contrast, once immune ozone was initiated again, the TBIL level began to decline again (Figure 5).

In conclusion, a patient with ACLF and AIHA responded well to hepato-protective drugs combined with immune ozone. Naturally, the effectiveness and safety of immune ozone in such patients should be further verified in large-scale randomized controlled trials.

References

1. Sarin SK, Kedarisetty CK, Abbas Z, *et al*. Acute-on-chronic liver failure: Consensus recommendations of the Asian Pacific Association for the Study of the Liver (APASL) 2014. Hepatol Int. 2014; 8:453-471.
2. Liver Failure and Artificial Extracorporeal Liver Support Group, Chinese Society of Infectious Diseases, Chinese Medical Association; Severe Liver Diseases and Artificial Extracorporeal Liver Support Group, Chinese Society of Hepatology, Chinese Medical Association. Diagnostic and treatment guidelines for liver failure (2012 ed.). Zhonghua Gan Zang Bing Za Zhi. 2013; 21:177-183. (in Chinese).
3. Liebman HA, Weitz IC. Autoimmune Hemolytic Anemia. Med Clin North Am. 2017; 101:351-359.
4. Böttiger LE, Westerholm B. Acquired haemolytic anaemia. I. Incidence and aetiology. Acta Med Scand. 1973; 193:223-226.
5. Hill QA. Autoimmune hemolytic anemia. Hematology. 2015; 20:553-554.
6. Red Blood Cell Disease (Anemia) Group of the Chinese Society of Hematology of the Chinese Medical Association. Chinese expert consensus on the diagnosis and treatment of autoimmune hemolytic anemia (2017). Zhonghua Xue Ye Xue Za Zhi. 2017; 38:265-267. (in Chinese)
7. Smith NL, Wilson AL, Gandhi J, Vatsia S, Khan SA. Ozone therapy: An overview of pharmacodynamics, current research, and clinical utility. Med Gas Res. 2017; 7:212-219.
8. Bocci V, Zanardi I, Travagli V. Ozone: A new therapeutic in vascular diseases. Am J Cardiovasc Drugs. 2011; 11:73-82.
9. Sagai M, Bocci V. Mechanisms of action involved in ozone therapy: Is healing induced *via* a mild oxidative stress? Med Gas Res. 2011; 1:29.
10. Bocci V, ed. Ozone: A new medical drug. 2 ed: Springer Netherlands; 2011.
11. Danan G, Teschke R. RUCAM in drug and herb induced liver injury: The update. Int J Mol Sci. 2015; 17:pii: E14.
12. Okazaki K, Uchida K, Koyabu M, Miyoshi H, Ikeura T, Takaoka M. IgG4 cholangiopathy: Current concept, diagnosis, and pathogenesis. J Hepatol. 2014; 61:690-695.
13. Inal M, Dokumacioglu A, Özcelik E, Ucar O. The effects of ozone therapy and coenzyme Q10 combination on oxidative stress markers in healthy subjects. Ir J Med Sci. 2011; 180:703-707.
14. Mandzhgaladze NR, Kharebava ER, Didia TsG, Ardzhevanishvili MD, Gudzhabidze MV, Chigiashvili Ts N. Influence of intravenous ozone treatment on the level of different specificity antibodies. Georgian Med News. 2006; 138:93-95.

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