CASE REPORT

Acute Kidney Injury by Renal Hemosiderosis Secondary to Primary Cold Agglutinin Disease Associated with an Excessive Alcohol Intake

Aya Imafuku1, Go Yamamoto2, Koji Takemura1, Eiko Hasegawa1, Naoki Sawa1, Masahiro Kawada1, Akinari Sekine1, Junichi Hoshino1, Kenmei Takaichi1, Takeshi Fujii3, Kenichi Ohashi4 and Yoshifumi Ubara1,5

Abstract:
Renal hemosiderosis occurs in the context of severe intravascular hemolysis, with the most common cause being paroxysmal nocturnal hematuria. Patients with cold agglutinin disease (CAD) have relatively mild hemolysis, and acute kidney injury (AKI) due to renal hemosiderosis has not been reported. We encountered a patient with CAD caused by lymphoplasmacytic lymphoma who developed AKI secondary to renal hemosiderosis after an excessive alcohol intake.

Key words: acute kidney injury, renal hemosiderosis, intravascular hemolysis, cold agglutinin disease, lymphoplasmacytic lymphoma, excessive alcohol intake

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Introduction

Renal hemosiderosis occurs as a consequence of severe intravascular hemolysis. The majority of patients with renal hemosiderosis have paroxysmal nocturnal hematuria (PNH) (1, 2), and only a few cases have been reported due to other causes, such as prosthetic heart valves, ABO-incompatible transfusion, sickle cell anemia, or primary hemochromatosis (3-5).

Primary cold agglutinin disease (CAD) accounts for 15% of autoimmune hemolytic anemia (6), and half of these patients have lymphoplasmacytic lymphoma/Waldenstrom’s macroglobulinemia (LPL/WM) as the underlying cause (7). Hemolysis is usually mild in CAD and has never been reported to cause renal hemosiderosis (8).

However, we encountered a patient with CAD and LPL/WM who developed acute kidney injury (AKI) caused by renal hemosiderosis due to severe intravascular hemolysis.

Case Report

A 67-year-old man was admitted to our hospital with malaise and jaundice in December 2013. He was a heavy drinker and had been drinking much more than usual for one week prior to admission. For the past three years, he had noted Raynaud’s phenomenon and dark urine after exposure to cold. The results of his previous annual medical checkups were unremarkable, except for an elevation of total bilirubin to 2.0-3.5 mg/dL.

On admission, his blood pressure was 132/73 mmHg, pulse rate was 100/min, and body temperature was 39.5°C. A physical examination showed conjunctival pallor and severe jaundice, but there was no abdominal tenderness, splenomegaly, or lymphadenopathy. Laboratory tests revealed liver dysfunction, renal dysfunction, and hemolytic anemia (Table 1): GOT, 302 U/L; GPT, 266 U/L; gamma-glutamyl transpeptidase (γ-GTP), 440 U/L; alkaline phos-
Table 1. Laboratory Tests Revealed Liver Dysfunction, Renal Dysfunction, and Hemolytic Anemia.

| Blood tests | Normal range | Normal range |
|-------------|--------------|--------------|
| WBC 9,300 /μL | 3,400-9,200 | CRP 6.4 mg/dL | 0.0-0.3 |
| Seg 84.5 % | 45.6-73.2 | Haptoglobin <10 mg/dL |
| Eos 0.0 % | 0.6-8.4 | Direct Coombs test + |
| Lym 12.5 % | 19.0-45.4 | Anti-complement antibody + |
| RBC 271×10^6 /μL | 400-566×10^6 | Cold agglutinins 65,536 |

| All 13.0-17.0 | IgG 1,395 mg/dL | 870-1,700 |
| Pti 25.1×10^4 /μL | 14.1-32.7 | IgA 298.1 mg/dL | 110-410 |
| TP 7.4 g/dL | 6.9-8.4 | IgM 282.6 mg/dL | 35-220 |
| Alb 4.3 g/dL | 3.9-5.2 | IgM-κ protein + |
| GOT 302 U/L | 13-33 | CH50 25 U/mL | 30-50 |
| GPT 266 U/L | 8-42 | C3 80 mg/dL | 6-160 |
| LDH 1,148 U/L | 119-229 | C4 7 mg/dL | 17-45 |
| ALP 310 U/L | 117-350 | Antinuclear antibody <40 |
| γ-GTP 440 U/L | 9-109 | Rheumatoid factor 6 IU/mL | 0-15 |
| T-Bil 45.1 mg/dL | 0.3-1.1 | Anti-M2 antibody <1.5 IU |
| D-Bil 30.1 mg/dL | 0.0-0.2 | MPO-ANCA <10 IU |
| UN 24 mg/dL | 8-12 | PR3-ANCA <10 IU |
| Cr 1.39 mg/dL | 0.65-1.06 | Cryoglobulin - |
| eGFR 40.5 mL/min | Soluble IL2 receptor 345 U/mL | 145-519 |
| Na 138 mEq/L | 139-146 | Total cholesterol 146 mg/dL | 122-240 |
| K 4.5 mEq/L | 3.7-4.8 | Triglyceride 77 mg/dL | 30-150 |
| Fe 198 μg/dL | 80-120 | Cholinesterase 186 IU/L | 220-495 |
| TIBC 263 μg/dL | 253-383 | Prothrombin time 62.5 % >75 |
| Ferritin 2,172 μg/L | 10-190 | APTT 22.5 s | 25-36 |

| Urine tests | Normal range | Normal range |
|-------------|--------------|--------------|
| Protein 3.0 g/gCr | <0.15 | Bilirubin + |
| RBC 1-4 HPF | <1 | Urobilinogen 2+ |
| BLP-κ | + | 1+ |

Figure 1. Non-contrast computed tomography showed no significant changes in the liver or biliary tract.

phatase (ALP), 310 U/L; lactate dehydrogenase (LDH), 1148 U/L; total bilirubin (T-Bil), 45.1 mg/dL; direct bilirubin (D-Bil), 30.1 mg/dL; serum creatinine, 1.39 mg/dL; blood urea nitrogen, 24 mg/dL; urine protein, 3.0 g/gCre; urine blood (2+) with 1-4 red blood cells per high-power field; urine bilirubin (3+); and urine urobilinogen (2+). His hemoglobin was 9.2 g/dL, and haptoglobin was <10 mg/dL. The direct Coombs test and anti-complement antibody were both positive, and the cold agglutinin titer was 65,536. No evidence of infection was identified by blood cultures, viral serology (for hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis E virus, Epstein-Barr virus, cytomegalovirus, and human immunodeficiency virus), or testing for Mycoplasma. Accordingly, primary CAD was diagnosed. Non-contrast computed tomography showed no significant changes in the liver or biliary tract. The kidney size was 11 cm, with no hydronephrosis in either kidney (Fig. 1). Mag-

Figure 2. T2-weighted magnetic resonance imaging showed no specific signal intensity change in the kidneys.
Figure 3. Kidney pathology. (a) Hematoxylin and Eosin staining shows acute tubular necrosis. (b) Periodic acid-Schiff staining shows brown pigment in the proximal tubular epithelial cells (arrowhead). (c) Prussian blue staining shows numerous hemosiderin deposits in the proximal tubular epithelial cells. (d) Electron microscopy shows granular hemosiderin deposits in the lysosomes of proximal tubular epithelial cells.

Magnetic resonance cholangiopancreatography also showed no evidence of biliary obstruction and no specific signal changes in the kidneys (Fig. 2).

After admission, his liver function improved spontaneously, but anemia progressed, and the renal function also deteriorated, with the serum creatinine rising to 6.3 mg/dL on hospital day 2. At this time, we detected positive serum IgM-κ protein and urine Bence-Jones protein (BJP)-κ. Therefore, we performed a bone marrow biopsy and kidney biopsy on day 3.

**Bone marrow biopsy findings**

The bone marrow smear contained 1.4% large lymphoid cells and 0.6% plasma cells. Flow cytometry showed that the lymphoid cells were positive for CD5dim, CD19, CD20, CD22, CD23, and BCL2 and negative for CD10 and CyclinD1 along with light chain restriction (κ>λ), suggesting monoclonal proliferation of B cells. Erythrocytes did not express CD55 or CD59, excluding the possibility of PNH. These findings were compatible with a diagnosis of LPL/WM.

**Kidney biopsy findings**

The biopsy specimen contained 51 glomeruli. Only one glomerulus showed global sclerosis, and there were no significant changes in the other glomeruli. On hematoxylin and eosin staining, there was epithelial thinning, loss of the brush border, and detachment of epithelial cells in the proximal tubules, indicating acute tubular necrosis (Fig. 3a). In sections subjected to periodic acid-Schiff staining, deposits of brown pigment were seen in the proximal tubules (Fig. 3b), and Prussian blue staining revealed numerous hemosiderin deposits in the proximal tubular epithelial cells (Fig. 3c). Immunofluorescence was negative for immune deposits. Electron microscopy revealed granular hemosiderin deposits in the lysosomes of proximal tubular epithelial cells (Fig. 3d). Thus, the final diagnosis was AKI caused by renal hemosiderosis that was secondary to CAD due to underlying LPL/WM.

**Clinical course**

Fig. 4 summarizes the patient’s clinical course. After the diagnosis of CAD, treatment with dexamethasone was started on hospital day 3 (40 mg/day for 4 days), and plasma exchange was initiated from day 4. We used fresh-frozen plasma as replacement solution, and the average volume of treated plasma was 3,400 mL for each session. However, his kidney function deteriorated rapidly on day 4 (with oliguria and serum creatinine of 7.2 mg/dL), requiring hemodialysis. Hemolysis improved rapidly after the initiation of dexamethasone therapy and plasma exchange, and kidney dysfunction also improved accordingly. Hemodialysis was withdrawn on hospital day 9 (after 3 sessions), and plasma exchange was withdrawn on day 11 (after 4 sessions). He started treatment with rituximab (375 mg/m² weekly) for LPL/WM on day 14 and received a total of 4 doses. The patient was discharged on hospital day 35 with a serum creatinine level of 1.0 mg/dL, hemoglobin of 7-8 g/dL, and total bilirubin of 2 mg/dL. Four years later, he is doing well without any treatment, but with a reduced alco-
hol intake and avoidance of cold exposure (Table 2).

**Discussion**

This is the first report of AKI caused by renal hemosiderosis in a patient with CAD and LPL/WM.

PNH is well known as the main cause of renal hemosiderosis (1, 2), which leads to AKI and even chronic kidney disease in some patients (2, 9). However, few reports have described AKI secondary to renal hemosiderosis in patients with other hemolytic diseases. The mechanism underlying renal hemosiderosis has been reported as follows (10, 11): When hemolysis occurs, dimeric hemoglobin binds with haptoglobin in the plasma, after which haptoglobin-hemoglobin complexes are taken up and degraded by reticuloendothelial cells. However, plasma haptoglobin becomes saturated if there is massive hemolysis, allowing free dimeric hemoglobin to be filtered through the glomeruli and absorbed by the proximal tubules. In the tubular cells, hemoglobin dissociates into heme and globin, and heme proteins cause AKI through three mechanisms: 1) direct cytotoxicity, 2) decreased renal perfusion due to depletion of nitric oxide, and 3) cast nephropathy when casts are formed via the interaction of heme proteins with Tamm-Horsfall protein. Since we did not observe any cast formation, we believe that the kidney injury in the present case was caused mainly by direct cytotoxicity and decreased renal perfusion.

Magnetic resonance imaging is the only imaging modality to reveal hemosiderin deposition in the renal cortex in patients with PNH via the reversal of the normal cortical and medullary intensity on T1-weighted images (the cortex shows a lower signal intensity than the medulla) and with very low cortical intensity on T2-weighted images (12). In the present case, we did not note these typical changes on MRI findings. Renal hemosiderin deposition in the current case would have been milder than in patients with PNH who suffer from repeated episodes of severe hemolysis attack.

Among patients with autoimmune hemolytic anemia, 15% have primary CAD (6). A bone marrow biopsy reveals a clonal lymphoproliferative disorder in 75% of CAD patients, and half of these patients have LPL/WM (7). Conversely, 3% of patients with LPL/WM also have primary CAD (13). Raynaud’s phenomenon is induced by cold temperatures in 90% of CAD patients, and 75% also show exacerbation of hemolysis with febrile illnesses (7). The median hemoglobin...
is reported to be 9.2 g/dL (4.5-15.6), and the median total bilirubin level is 2.4 mg/dL (0.6-9.5) (7). Thus, primary CAD is associated with relatively mild hemolysis, and there have been no reports of renal hemosiderosis or AKI in this disease. In the present patient, primary CAD was associated with atypically severe intravascular hemolysis, which resulted in AKI due to renal hemosiderosis. Although why this patient developed severe hemolysis is unclear, his excessive alcohol intake and alcoholic liver dysfunction may have been involved. An excessive alcohol intake is known to trigger hemolysis in patients with PNH (14), and a chronic excessive alcohol consumption affects the structural integrity of red blood cell membranes due to increased oxidative stress (15, 16). Furthermore, hepatic synthesis of haptoglobin and processing of heme proteins might have been impaired in this patient due to his severe alcoholic liver dysfunction.

In conclusion, primary CAD can be associated with severe hemolysis, resulting in AKI due to renal hemosiderosis, especially in patients with an excessive alcohol intake.

The authors state that they have no Conflict of Interest (COI).

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References

1. Gelfand JA, Frank MM. Letter: renal failure in paroxysmal hemo-globulinuria. JAMA 227: 440, 1974.
2. Chow KM, Lai FM, Wang AY, Chan YL, Tang NL, Li PK. Reversible renal failure in paroxysmal nocturnal hemoglobinuria. Am J Kidney Dis 37: E17, 2001.
3. Qian Q, Nath KA, Wu Y, Daoud TM, Sethi S. Hemolysis and acute kidney failure. Am J Kidney Dis 56: 780-784, 2010.
4. Calazans LM, de Souza, Santos RF, de Souza, Goncalves M, Dos-Santos WL, Rocha PN. Renal hemosiderosis complicating sickle cell anemia. Kidney Int 81: 709, 2012.
5. Ozkurt S, Acikalin MF, Temiz G, Akay OM, Soydan M. Renal hemosiderosis and rapidly progressive glomerulonephritis associated with primary hemochromatosis. Ren Fail 36: 814-816, 2014.
6. Sokol RJ, Hewitt S, Stamps BK. Autoimmune haemolysis: an 18-year study of 865 cases referred to a regional transfusion centre. Br Med J (Clin Res Ed) 282: 2023-2027, 1981.
7. Berentsen S, Ulvestad E, Langholm R, et al. Primary chronic cold agglutinin disease: a population based study of clinical cases. Scand J Haematol 91: 460-466, 2007.
8. Nydegger UE, Kazatchkine MD, Miescher PA. Immunopathologic and clinical features of hemolytic anemia due to cold agglutinins. Semin Hematol 28: 66-77, 1991.
9. Clark DA, Butler SA, Braren V, Hartmann RC, Jenkins DE Jr. The kidneys in paroxysmal nocturnal hemoglobinuria. Blood 57: 83-89, 1981.
10. Tracz MJ, Alam J, Nath KA. Physiology and pathophysiology of heme: implications for kidney disease. J Am Soc Nephrol 18: 414-420, 2007.
11. Rother RP, Bell L, Hillmen P, Gladwin MT. The clinical sequelae of intravascular hemolysis and extracellular plasma hemoglobin: a novel mechanism of human disease. JAMA 293: 1653-1662, 2005.
12. Mulpopulos GP, Turner DA, Schwartz MM, Murakami ME, Clark JW. MRI of the kidneys in paroxysmal nocturnal hemoglobinuria. AJR Am J Roentgenol 146: 51-52, 1986.
13. Dimopoulos MA, Hamilos G, Zervas K, et al. Survival and prognostic factors after initiation of treatment in Waldenström's macroglobulinemia. Ann Oncol 14: 1299-1305, 2003.
14. Pu JJ, Brodsky RA. Paroxysmal nocturnal hemoglobinuria from bench to bedside. Clin Transl Sci 4: 219-224, 2011.
15. Kok VC, Lee CK, Horng JT, Lin CC, Sung FC. Reappraisal of the etiology of extracorpuscular non-autoimmune acquired hemolytic anemia in 2657 hospitalized patients with non-neoplastic disease. Clin Med Insights Pathol 7: 11-14, 2014.
16. Bulle S, Reddy VD, Padmanabhi P, Muraru P, Puvvada PK, Nallanchakravartula V. Association between alcohol-induced erythrocyte membrane alterations and hemolysis in chronic alcoholics. J Clin Biochem Nutr 60: 63-69, 2017.

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