Hepatitis B virus-associated cryoglobulinaemia diffuse endocapillary proliferative glomerulonephritis: a case report and literature review

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Abstract
Cryoglobulinaemia can manifest as fatigue, purpura, and joint pain, and can involve the kidneys and peripheral nervous system. Type II and mixed cryoglobulinemia cases are usually associated with hepatitis C virus infection and autoimmune diseases, and most cases reported outside China have been related to hepatitis C virus. The pathological manifestation of cryoglobulinaemia glomerulonephritis is always membranous proliferative glomerulonephritis or membranous nephropathy; other pathological types are rare. This current case report describes a female patient with hepatitis B virus (HBV)-associated cryoglobulinaemic glomerulonephritis. The patient had hepatitis B complicated with purpura, abnormal urinalysis and renal function. She was positive for rheumatoid factor and had decreased complement, and her blood cryoglobulin level was positive. The pathological findings were consistent with late-stage capillary proliferative glomerulonephritis, which improved after steroid, immunosuppressant and anti-HBV treatment.

Keywords
Hepatitis B virus-associated cryoglobulinaemia, diffuse endocapillary proliferative glomerulonephritis

Date received: 21 February 2022; accepted: 9 September 2022

Introduction
Cryoglobulinaemia is the presence of abnormal immunoglobulins (Ig) in the serum that precipitate at low temperature and redissolve at higher temperature.¹ It is a systemic small vasculitis. The symptoms arise mainly from inflammation caused by
the deposition of circulating immune complexes, which affect mainly the skin, peripheral nervous system and kidneys. Renal damage is common and the main pathological type is membranous proliferative glomerulonephritis (MPGN). This case report describes a patient with hepatitis B virus (HBV)-associated cryoglobulinaemia diffuse endocapillary proliferative glomerulonephritis and it provides a literature review to improve the understanding of this disease.

Case report

On 2 June 2018, a 47-year-old woman with recurrent lower-extremity ecchymosis of more than 2 years duration was hospitalized in the Department of Rheumatology, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou City, Zhejiang Province, China. Previously, she had developed petechiae and ecchymosis on both feet and legs, with no obvious cause. These did not fade under pressure and were scattered in red-wine masses with no itching or pain; the rash subsided after 4–5 days, accompanied by knee pain. In April 2018, the rash decreased slightly after treatment with 48 mg methylprednisolone orally once a day. After reducing the methylprednisolone dose to 20 mg once a day, she developed watery stool with mucus seven to eight times a day. Her physician thought that the diarrhoea was related to the methylprednisolone and discontinued it. After drug withdrawal, the rash on both lower limbs worsened progressively. In June 2018, a skin biopsy of the left thigh showed positive angulation, no obvious epidermal abnormality, mononuclear lymphocyte infiltration around the blood vessels in the superficial dermis, a few neutrophils and some nuclear fragmentation, oedema of the blood vessels, and occasional eosinophils. As immunofluorescence markers were negative, it was considered to be urticaria vasculitis. Urinalysis results were as follows: occult blood, 3+; protein, 1+; 24-h urinary protein level, 951 mg; creatinine level, 66 μmol/l; estimated glomerular filtration rate (eGFR), 95.8 ml/min; negative for antinuclear antibody and anti-neutrophil cytoplasm antibodies; positive for hepatitis B surface antigen, e antibody and core-antibody positive; HBV-DNA level, $2.55 \times 10^5$ IU/ml; rheumatoid factor (RF) level, 799.1 IU/ml; IgG level, 553 mg/dl; IgM level, 61.4 mg/dl; IgA level, 97.2 mg/dl; complement 3 (C3) level, 56.3 mg/dl; and complement 4 (C4) level, 5.3 mg/dl.

In June 2018, a renal biopsy showed no thickening of the capillary basement membrane, obvious endothelial cell proliferation, mild mesangial hyperplasia or erythrophilic protein deposition. The renal tubular epithelial cells showed partial vacuolar degeneration, <5% tubular atrophy, some focal interstitial fibrosis, focal lymphocyte infiltration and no obvious arteriole wall thickening. Immunofluorescence scores were 2+ for IgA, IgM, and C1q, and 3+ for C3 (all along the mesangium), whereas IgG and C4 were negative. The diagnosis was endocapillary proliferative glomerulonephritis (Figure 1). She was given 80 mg methylprednisolone intravenous (i.v.) daily for 11 days. The dose of methylprednisolone was gradually reduced. The patient then received 0.5 mg entecavir orally once a day for 28 months, after which she improved somewhat. After the methylprednisolone had been gradually reduced to 24 mg orally once a day, her lower limbs developed petechiae and ecchymosis. As her Ig levels were low (66.3 mg/dl IgA, 185 mg/dl IgG, and 44.1 mg/dl IgM), she was given 20 g gamma globulin 20 i.v. once. After this, she developed fever, chills, muscle soreness, nausea, vomiting, soy-sauce coloured urine and a decreased platelet count. The gamma globulin treatment was stopped. She improved and was discharged after receiving 10 mg
chlorpheniramine intramuscular injection once and 10 mg metoclopramide intramuscular injection once. On follow-up, the blood cryoglobulin level was positive.

On 20 July 2019, she was readmitted because of repeated skin ecchymosis events. Physical examination on admission showed ecchymosis and a few petechiae on both legs, thighs and upper limbs; these areas were dark red and did not fade with pressure. There were pigmented spots on many parts of her body, without pruritus or pain. Laboratory examinations were as follows: urinalysis showed 2+ occult blood and protein, and 24-h urine tests showed 1231.8 mg protein, 111 μmol/l creatinine, 51.2 ml/min eGFR and negative HBV-DNA.

A repeat renal biopsy showed capsule wall fibrosis in three glomeruli and shrinkage of a few capillaries in the basement membrane. Some glomerular mesangial areas had moderate-to-severe hyperplasia with increased endothelial cells. There was no obvious capillary basement membrane thickening. There was multifocal atrophy in approximately 30% of the renal tubules; the tubular epithelial cells showed granular degeneration, and some tubules were low and flat. Transparent tubes were seen, some of which were thick. There was interstitial focal fibrosis with marked lymphocyte infiltration. Some arterioles were thickened and fibrotic. Immunofluorescence scores were 2+/3+ for C3, 2+ for Clq and 2+ for IgM (mesangial area and capillary basement membrane deposition), whereas IgA, C4 and IgG were negative. Immunohistochemistry showed positive pla2rl and negative IgG4.

The pathological findings were consistent with late-stage capillary proliferative glomerulonephritis (Figure 2). Electron microscopy showed a glomerulus with poor opening of the capillary loop, marked cell proliferation in the capillary loop, diffuse fusion of the foot process, increased mesangial matrix and no electron-dense deposits. Microthrombotic formation was seen within the capillary loop. The patient received 500 mg mycophenolate mofetil orally twice a day and 16 mg methylprednisolone orally once a day for 2 months. The methylprednisolone was stopped in April 2021 and the mycophenolate mofetil dose was reduced in May 2021. At the last follow-up on 23 June 2021, a few old ecchymosis spots were seen on both thighs and pigmented spots were seen on many parts of the body. The 24-h urinary protein level was 102 mg, creatinine was 68 μmol/l, eGFR was 103.0 ml/min and HBV-DNA was <30 IU/ml.

The study protocol was approved by the Ethics Committee of Zhejiang University School of Medicine Sir Run Run Shaw Hospital.
Cryoglobulin is an abnormal Ig that precipitates at 0–4°C and redissolves at 37°C. To test this, 20 ml blood is collected at 37°C and maintained at 4°C for 7 days; the amount of centrifugal sediment is then measured using the cold sedimentation specific volume method. Cryoglobulin can be divided into three types: (i) type I comprises monoclonal IgG, IgM and IgA, and is common in lymphoproliferative diseases such as multiple myeloma; (ii) type II is composed of monoclonal IgM-RF (or IgG or IgA) and polyclonal Igs (mainly IgG), and is common in infections, autoimmune diseases, and lymphoproliferative diseases; and (iii) type III comprises mainly polyclonal Igs and polyclonal Ig-RF (mainly IgM), and is seen mainly in hepatitis C virus (HCV) infections and autoimmune diseases. This current case was investigated using modified capillary immunotyping (SEBIA Company, Paris, France) or immunofixation electrophoresis for cryoglobulin identification. Cryoglobulin positivity was defined as a cryocrit level >1.0% in serum samples. Other biological features include cryoglobulinaemia can manifest as fatigue, purpura, and joint pain, and can involve the kidneys and peripheral nervous system. Type II and mixed cryoglobulinaemia are seen mainly in HCV infections and autoimmune diseases. Most reported
cases are related to HCV.\textsuperscript{10} However, there have been some reports focusing on HBV-related cryoglobulinaemia. For example, research in Italy have shown that patients with HBV infection accounted for only 3\% of patients with cryoglobulinaemia.\textsuperscript{11} In 2016, another study summarized 17 cases of HBV-related cryoglobulinaemia.\textsuperscript{12} Studies at different centres in China have focused on HBV-associated cryoglobulinaemia,\textsuperscript{13,14} especially cases with renal involvement.\textsuperscript{7} Some research has reported that the HBV-infection rate in patients with cryoglobulinaemia may be equal to or greater than the HCV-infection rate.\textsuperscript{15} Antiviral nucleoside analogues, including entecavir, adefovir dipivoxil and lamivudine, are safe and effective for treating patients with HBV-associated cryoglobulinaemia vasculitis.\textsuperscript{14} These patients require long-term oral antivirals.\textsuperscript{12,16} Plasma exchange, steroids and immunosuppressants can be used when there are severe complications, such as reduced renal function.\textsuperscript{14} This current patient was HBV-positive and the blood globulin level was positive. In our hospital, two patients have been diagnosed with HBV-associated cryoglobulinaemia diffuse endocapillary proliferative glomerulonephritis based on the pathology. Their condition improved after treatment with steroids, immunosuppressants, and entecavir, and they remained stable after stopping the steroids and immunosuppressants. As the survival of patients with renal involvement is significantly reduced,\textsuperscript{17} these patients require close follow-up.

The typical pathological type of cryoglobulinaemia glomerulonephritis is MPGN or membranous nephropathy, which is usually characterized by type I MPGN dominated by IgM deposition.\textsuperscript{7} Mesangial proliferative glomerulonephritis, membranous nephropathy, focal segmental glomerulosclerosis, fibrous or immunotactoid glomerulopathy, and thrombotic microvascular disease have also been reported.\textsuperscript{18,19} Some authors think that the affinity of circulating IgM $\kappa$ for the mesangium is important in the deposition of mixed cryoglobulinaemia in the glomerulus and causes systemic damage.\textsuperscript{20} Cryoglobulinaemia glomerulonephritis is accompanied by cell proliferation and monocyte infiltration in the capillaries.\textsuperscript{13} Rhodopsin deposition can be seen under glomerular endothelial cells and erythrophilic protein capillary cavities form platinum ear or microthrombotic structures, with basement membrane thickening.\textsuperscript{6,13} These changes were not seen in this current patient on light microscopy. Repeated renal biopsies showed secondary endocapillary proliferative glomerulonephritis.

Infectious diseases, mainly HCV infection, have been reported as the most common cause of cryoglobulinemia.\textsuperscript{6,21} Hepatitis B virus-associated cryoglobulinaemia diffuse endocapillary proliferative glomerulonephritis is rare.\textsuperscript{10} Without a biopsy, skin purpura is easily misdiagnosed as renal damage caused by Henoch–Schonlein purpura. Therefore, cold globulinaemia should be considered in patients with hepatitis B complicated with purpura, abnormal urinalysis and renal function, positive RF, and decreased complement, and blood globulin should be tested. Patients with renal impairment should undergo a renal biopsy for further confirmation.\textsuperscript{13}

The current patient had a haemolytic reaction after gamma globulin treatment. Intravenous human gamma globulin (IVIg) was originally used as an alternative treatment for primary immunodeficiency disease. Since the first report that high-dose Ig can rapidly improve platelet numbers in patients with idiopathic thrombocytopenic purpura,\textsuperscript{22} its application has broadened. Muscle pain, headache, fever and discomfort are common adverse reactions of IVIg treatment.\textsuperscript{23} Serious adverse reactions include renal failure, aseptic meningitis, thromboembolism, and haemolytic
and allergic reactions. IVIg is a blood product and its main component is protein, more than 95% of which is Ig, including IgG antibodies against a broad spectrum of viruses, bacteria and other pathogens. Since plasma products can contain blood-group antibodies (e.g. anti-A, anti-B, anti-D), they can act as a haemolysin and induce red blood cell aggregation in the recipient, resulting in a positive direct antiglobulin test and haemolysis. Acute haemolysis has been reported during IVIg treatment; and the patients had serum antibodies against blood-group antigens; and some patients, mainly those with a non-O blood group, had haemolytic reactions during IVIg infusion. The current patient’s blood was type A, consistent with these findings.

In conclusion, the current case was a female patient with HBV-associated cryoglobulinaemic glomerulonephritis. The patient’s condition was complicated with purpura, abnormal urinalysis and renal function. The late-stage capillary proliferative glomerulonephritis improved after steroid, immunosuppressant and anti-HBV treatment.

Acknowledgement
We thank the patient for participating in this study.

Author contributions
Weiying Xu and Yongmei Han collected and analysed the clinical data and undertook the follow-up interview. Weiying Xu wrote and edited the manuscript. Yongmei Han provided guidance and reviewed the manuscript. Both authors contributed to the article and approved the submitted version.

Declaration of conflicting interests
The authors declare that there are no conflicts of interest.

Funding
The authors disclosed receipt (pending publication) of the following financial support for the research, authorship, and/or publication of this article: This case report was supported by a grant from the Health Commission of Zhejiang Province (no. 2019ZD043).

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