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Hepatitis B virus-related cryoglobulinemia: Clinical characteristics, virological features, and treatment

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ABSTRACT

Background: Hepatitis B virus (HBV) infection is a rare etiology of cryoglobulinemia, and its clinical characteristics, virological features and treatment are poorly understood.
Methods: This retrospective study enrolled 23 patients with HBV-related cryoglobulinemia from 497 cryoglobulinemia patients at Peking Union Medical College Hospital between January 2015 and February 2023. We analyzed the clinical characteristics, virological features and management of patients with HBV-related cryoglobulinemia.
Results: The 23 patients (13 males; median age 48 years) were all mixed cryoglobulinemia and serological HBsAg positive, while 15 patients exhibited HBV-DNA replication. The presence of HBsAg in cryoglobulins was evaluated in 7 patients, all of whom were positive. The most commonly involved organs were kidneys (69.6%), skin (65.2%), peripheral nerves (21.7%), joints (8.7%), gastrointestinal tract (4.3%), and cardiac (4.3%). Eight patients received antiviral therapy with nucleot (s)ide analogues (NAs) alone, 12 patients received NA- and corticosteroid-based regimens, and 3 patients received NA- and rituximab-based regimens based on the severity of clinical symptoms. After a median follow-up of 44 months, four patients died, and one patient was lost to follow-up. All remaining patients (n = 18) achieved clinical remission, and HBV-DNA replication was not detected in 16 out of 18 patients. There was no HBV reactivation in patients treated with rituximab. The three-year overall survival and progression-free survival were 87.0% and 80.3%, respectively.
Conclusions: HBV-related cryoglobulinemia patients should be treated with antiviral therapy. Corticosteroids and rituximab are effective for severe cases, but patients need to be closely monitored for therapy-related infection.

1. Introduction

Cryoglobulinemia is defined as the presence of cryoglobulins in the serum, which are immunoglobulins that precipitate at temperatures lower than 37 °C and redissolve upon rewarming (Ramos-Casals et al., 2012). Type II cryoglobulin is characterized by monoclonal immunoglobulin with rheumatoid factor (RF) activity and polyclonal immunoglobulin, and type III cryoglobulin is characterized by polyclonal immunoglobulin with RF activity (Brouet et al., 1974). The two types of cryoglobulins are also named as mixed cryoglobulins. Mixed cryoglobulinemia is usually associated with infections, mainly hepatitis C virus (HCV) infection, in up to 90% of patients (Saadoun et al., 2006), with geographic variations. Other common underlying diseases include hepatitis B virus (HBV) infection, human immunodeficiency virus (HIV) infection, connective tissue diseases (CTDs), and lymphoproliferative disorders (Ramos-Casals et al., 2012).

HBV infection affects approximately 350 million people worldwide, with wide geographical variations; for example, there is a higher prevalence of HBV infection in central Asia, especially in China. (Ganem and Prince, 2004) HBV infection is associated with a wide spectrum of liver diseases ranging from acute or fulminant hepatitis to chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. (Ganem and Prince, 2004) Furthermore, 20% of HBV patients may have extrahepatic manifestations, such as polyarthritis nodosa, glomerulonephritis and arthritis (Trépo et al., 2014). Cryoglobulinemia has been described in approximately 1.2–4% of HBV-infected patients (Ferri et al., 2004; Mazzaro

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et al., 2018; Terrier et al., 2015).

The relationship between cryoglobulinemia and HBV infection was first described in 1977. (Levo et al., 1977) The symptoms of HBV-related cryoglobulinemia patients vary from purpura and peripheral neuropathy to skin ulcer and glomerulonephritis. (Mazzaro et al., 2023) Because of its rarity, there is a lack of definitive guidelines on treatment. Although China has the largest population of individuals with HBV infection, accounting for one-third of infected individuals in the world, (Chen et al., 2018) there are still few studies focusing on HBV-related cryoglobulinemia.

Here, we performed a retrospective study to describe the clinical manifestations and virological features of HBV-related cryoglobulinemia, and we assessed the different treatments and outcomes of affected patients.

2. Materials and methods

2.1. Patients

This retrospective study was performed among patients with cryoglobulinemia at Peking Union Medical College Hospital between January 2015 and February 2023. The inclusion criteria were as follows: i) detectable cryoglobulins in the serum; ii) evidence of chronic HBV infection, including positivity for HBV surface antigen (HBsAg) in the serum, and/or detectable HBV-DNA in the serum, and/or positivity for HBsAg in the cryoglobulins; iii) clinical symptoms related to cryoglobulins; iv) exclusion of other secondary causes of cryoglobulinemia, such as HCV infection, HIV infection, CTDs, and hematological diseases. Informed consent was obtained from all patients, and the protocol was approved by the Peking Union Medical College Hospital Ethics Committee. The study was performed in accordance with the ethical standards of the 1964 Declaration of Helsinki and its later amendments.

2.2. Cryoglobulin and virological testing

The process of testing for cryoglobulins was in accordance with previous reports. (Motyckova and Murali, 2011) Peripheral blood samples were initially kept at 37 °C. After warm clotting and centrifugation, the serum was incubated at 4 °C for 7 days for cryoprecipitate formation. The serum sample was then washed and rewarmed to 37 °C. All samples positive for cryoglobulins were analyzed by immunoelectrophoresis to identify the type of cryoglobulin according to Brouet's criteria. (Brouet et al., 1974) Cryoglobulin quantification was performed in Wintrobe tubes to determine the cryocrit (packed volume of the precipitate relative to the original serum volume) and directly quantify immunoglobulins in combination with agarose gel electrophoresis.

We reviewed HBsAg and serum HBV-DNA quantification results. Both the HBsAg of the serum and cryoglobulins were tested using the chemiluminescence microparticle immunoassay (CMIA, Abbott) by Architect i2000SR (Abbott) according to the manufacturer's instructions, and HBsAg positivity was defined as $> = 0.05$ international units (IU)/ml. HBV-DNA was measured using real-time quantitative PCR by the Cobas TaqMan HBV Test version 2.0 (Roche Molecular Systems) with a low detection limit of 20 IU/ml or the DaAn Gene (China) RealTime HBV assay with a low detection limit of 1000 copies/ml.

2.3. Clinical and laboratory data

Clinical data were collected, including demographic characteristics, initial clinical manifestations, comorbid medical conditions, renal pathology, virological features and laboratory test results. Symptoms related to cryoglobulinemia were consistent with those of a previous study. (Zhang et al., 2020)

Laboratory data included the complete blood count, liver and kidney function parameters, urinalysis results, serum concentrations of the complement 3 (C3) and complement 4 (C4) fractions, the level of serum RF, serum/urine immunofixation electrophoresis (IFE) results, serum protein electrophoresis (SPE) results and 24-hour urine protein.

2.4. Treatment and outcomes

The therapies administered in this cohort included i) antiviral therapy with nucleot (s)ide analogues (NAs) alone, including entecavir (ETV) or lamivudine (LMV); ii) antiviral therapy with NAs and corticosteroid-based regimens; and iii) antiviral therapy with NAs and rituximab-based regimens. The response to treatment was consistent with that of a previous study and defined as clinical remission and a laboratory response. (Mazzaro et al., 2016; Zhang et al., 2020) Overall survival (OS) was calculated from the time of the diagnosis of cryoglobulinemia to the date of death or last follow-up. Progression-free survival (PFS) was calculated from the date of the diagnosis of cryoglobulinemia until the date of cryoglobulinemia progression and death from any cause or last follow-up. The last follow-up was February 28, 2023.

2.5. Statistical analysis

Continuous variables are presented as the median and range, and categorical variables are presented as frequencies and proportions. Data comparisons between two groups were performed using the Mann–Whitney U test for continuous variables and Fisher's exact test for categorical variables. Kaplan–Meier analysis was applied for survival analysis, and the survival curves were compared using the log-rank test. SPSS 26.0 software (IBM Corp., Armonk, NY, USA) was used for all analyses, and $P < 0.05$ was considered statistically significant.

3. Results

3.1. Patient characteristics

A total of 497 patients were diagnosed with cryoglobulinemia at Peking Union Medical College Hospital between January 2015 and February 2023. Overall, 23 (4.6%) patients (13 males and 10 females) were enrolled in this study. The baseline characteristics of all patients are summarized in Table 1. Patients with HBV-related cryoglobulinemia included 16 with type II cryoglobulinemia, including 15 with IgMκ and 1 with IgAλ, and 7 with type III cryoglobulinemia. The median age at diagnosis was 48 years old (range, 29–73 years), and the median duration from the diagnosis of HBV infection to cryoglobulinemia diagnosis was 192 months (range, 2–372 months). All patients with HBV-related cryoglobulinemia were serological HBsAg positive as well as HCV-antibody-negative, and 15 patients (65.2%) exhibited HBV-DNA replication. Furthermore, we performed tests for HBsAg in cryoglobulins among 7 patients (30.4%), and all patients were positive.

We compared the baseline characteristics of HBV-related cryoglobulinemia patients with HBsAg positivity in cryoglobulins with those of patients for whom HBsAg in cryoglobulins was not evaluated (Table 2). Of all patients, the median number of involved organs was 2 (range 1–4). The frequency and distribution of the cryoglobulinemia-related symptoms in all patients are shown in Fig. 1. The most common symptoms included proteinuria (69.6%); purpura (65.2%); renal function impairment (43.5%), including 2 patients who presented with rapidly progressive glomerulonephritis (RPGN); hematuria (39.1%); peripheral neuropathy (21.7%); arthralgia (8.7%); livedo reticularis (8.7%); cutaneous ulcer (4.3%); diarrhea and hematochezia (4.3%); and dyspnea as well as decreased exercise tolerance (4.3%). The most commonly involved organs/tissues were the kidneys (69.6%), followed by the skin (65.2%), peripheral nerves (21.7%), joints (8.7%), gastrointestinal (GI) tract (4.3%), and cardiac system organs/tissues (4.3%). We did not find any significant differences in organ involvement or clinical symptoms related to cryoglobulinemia between patients with HBsAg positivity in cryoglobulins and those without the evaluation of

Table 1
Demographics and clinical characteristics of all HBV-related cryoglobulinemia patients.

Patient	Sex, Age	HBsAg in CGs, IU/ml	Involved organ	HBV-DNA	ALT, U/L	Cryoglobulin Detection			Treatments and Outcomes
						Isotype	Cryocrit (n,%)	Cryoglobulin level, mg/L	
#1	Female 57 y	28.71	Skin, peripheral nerves, kidney	Neg	11	Type II (IgMκ)	23.0	10,925.0	ETV+CS+CTX+PE: CR[a], PR[b], then relapse[c], PFS 32 m ETV+CS+CTX+PE: NR[a, b] ETV+R+CS: PR[a], NR[b] Death from covid-19 infection
#2	Female 41 y	49.7	Skin, kidney	52 IU/ml	10	Type II (IgMκ)	5.0	1112.5	ETV+R-CP, ETV+R-P: CR[a], PR[b] HBV-DNA undetected
#3	Male 29 y	1608.8	kidney, heart	2.37×10^5	31	Type II (IgMκ)	4.0	687.3	ETV+CS: PR[a, b], HBV-DNA undetected, then relapse[c], PFS 41 m, HBV-DNA 2.0 × 10^4 ETV+CS: PR[a]
#4	Male 39 y	0.11	Skin, peripheral nerves	Neg	27	Type II (IgMκ)	2.5	1000.9	ETV+RTX: CR[a], PR[b]
#5	Female 60 y	22.2	Skin	2.62×10^3	20	Type II (IgMκ)	1.5	858.5	ETV: CR[a], PR[b] HBV-DNA undetected
#6	Female 45 y	75.37	Skin, peripheral nerve, kidney, GI tract	Neg	12	Type II (IgMκ)	5.0	3840.0	ETV+Rd+PE: NR[a, b] Death from RPGN (cryoglobulinemia-related event)
#7	Male 50 y	Pos	Skin, kidney	1.18×10^4	29	Type III	<1.0	62.2	ETV+CS Loss of follow-up
#8	Female 47 y	NA	Skin, joint, kidney	Neg	21	Type II (IgMκ)	1.0	NA	ETV+CS+MMF: PR[a]
#9	Female 48 y	NA	Skin, peripheral nerves	4.11×10^5	28	Type II (IgAλ)	17.0	NA	ETV+CS+MMF: CR[a], CR[b] HBV-DNA undetected
#10	Female 55 y	NA	Skin, joint	1.61×10^5	15	Type II (IgMκ)	4.0	NA	ETV: CR[a], PR[b], HBV-DNA undetected, then relapse[c], PFS 55 m, HBV-DNA 5.30×10^3 ETV: PR[a], PR[b]
#11	Male 67 y	NA	Skin, kidney	Neg	20	Type II (IgMκ)	4.0	NA	LMV+CS+CTX: NR[a] Death from infection
#12	Male 53 y	NA	Skin, kidney	Neg	20	Type II (IgMκ)	<1.0	78.4	ETV+CS: NR[a] Death from infection
#13	Female 38 y	NA	kidney	1.70×10^8	101	Type II (IgMκ)	15.0	8012.2	ETV+CS+PE: CR[a], PR[b], HBV-DNA undetected, ALT 32 IU/ml
#14	Female 37 y	NA	Skin, kidney	4.40×10^5	35	Type II (IgMκ)	1.0	225.3	ETV: CR[a], CR[b] HBV-DNA undetected
#15	Male 41 y	NA	Kidney	28.2 IU/ML	30	Type II (IgMκ)	1.0	43.1	ETV+CS+CTX: CR[a] HBV-DNA undetected
#16	Male 64 y	NA	Kidney	9.12×10^3	59	Type II (IgMκ)	<1.0	124.3	ETV: PR[a] HBV-DNA undetected
#17	Male 38 y	NA–	peripheral nerves	1.49×10^5	68	Type II (IgMκ)	11	1363.9	ETV: PR[a] HBV-DNA 1.0 × 10^3
#18	Male 60 y	NA	Kidney	Neg	20	Type III (polyIgM)	<1.0	24.9	ETV+CS: PR[a]
#19	Male 38 y	NA	Kidney	Neg	44	Type III (polyIgM+IgG)	<1.0	64.1	ETV+CS: PR[a], CR[b]
#20	Male 66 y	NA	Skin, kidney	684IU/ml	77	Type III	<1.0	16.4	ETV+CS: PR[a], HBV-DNA undetected, ALT 37 IU/ml
#21	Female 33 y	NA	Kidney	1.79×10^5	17	Type III	<1.0	42.3	ETV: PR[a] HBV-DNA 2.0 × 10^3
#22	Male 59 y	NA	Skin	6.93×10^5	41	Type III (polyIgM+IgA)	1.0	164.0	ETV: CR[a] HBV-DNA undetected
#23	Male 73 y	NA	Skin	5.20×10^4	16	Type III (polyIgM+IgG)	1.0	182.7	ETV: PR[a] HBV-DNA undetected

Ccr = endogenous creatinine clearance rate; CGs = cryoglobulins; CR = complete response; CS = corticosteroids; CTX = cyclophosphamide; ETV = entecavir; GI = gastrointestinal; LMV = lamivudine; MMF = mycophenolate mofetil; Neg = negative; NR = no response; PE = plasma exchange; PR = partial response; Pos = positive; R = rituximab; R-CP = rituximab, cyclophosphamide, and prednisone; Rd = rituximab and dexamethasone; R-P = rituximab and prednisone; RPGN = rapidly progressive glomerulonephritis; – = no evaluation for HBsAg in CGs.

[a] clinical remission.

[b] laboratory response.

[c] cryoglobulinemia progression.

HBsAg in cryoglobulins.

3.2. Laboratory findings and virological features

At diagnosis, six patients (26.1%) showed abnormal liver function, and the median alanine aminotransferase (ALT) level was 64 U/L (range, 41–101 U/L). All patients with kidney involvement presented with proteinuria, and the median 24-hour urine protein level was 2.05 g/L (0.55–6.80 g/L), while 9 (56.3%) patients experienced hematuria. Among the patients with renal function impairment, the median serum creatinine (sCr) level and endogenous creatinine clearance rate level were 199 μmol/L (range, 106–322 μmol/L) and 41.46 ml/min/1.73 m^2

Table 2
Clinical characteristics of HBV-related cryoglobulinemia patients with HBsAg positivity in cryoglobulins and those of patients for whom HBsAg presence in cryoglobulins was not evaluated.

Clinical characteristics	All patients (n = 23)	Patients with HBsAg pos in CGs (n = 7)	Patients for whom HBsAg presence in CGs was not evaluated (n = 16)	P value
Male (n,%)	13 (56.5)	3 (42.9)	10 (62.5)	0.650
Age, years, median (range)	48 (29–73)	45 (29–60)	51 (33–73)	0.359
Skin involvement (n,%)	15 (65.2)	6 (85.7)	9 (56.3)	0.345
Purpura (n,%)	15 (65.2)	6 (85.7)	9 (56.3)	0.345
Livedo reticularis (n,%)	1 (4.3)	0 (0.0)	1 (6.3)	–
Ulcers (n,%)	1 (4.3)	1 (14.3)	0 (0.0)	–
Peripheral nerves involvement (n,%)	5 (21.7)	3 (42.9)	2 (12.5)	0.142
Arthralgia (n,%)	2 (8.7)	0 (0.0)	2 (12.5)	–
Renal involvement (n,%)	16 (69.6)	5 (71.4)	11 (68.8)	1.000
Proteinuria (n,%)	16 (69.6)	5 (71.4)	11 (68.8)	1.000
Hematuria (n,%)	9 (39.1)	3 (42.9)	6 (37.5)	1.000
Renal impairment (n,%)	10 (43.5)	4 (57.1)	6 (37.5)	0.650
Cardiac involvement (n,%)	1 (4.3)	1 (14.3)	0 (0.0)	–
GI tracts involvement (n,%)	1 (4.3)	1 (14.3)	0 (0.0)	–
Type II cryoglobulinemia, n (%)	16 (69.6)	6 (85.7)	10 (62.5)	0.366
IgMκ, n (%)	15 (65.2)	6 (85.7)	9 (56.3)	0.345
IgAλ, n (%)	1 (4.3)	0 (0.0)	1 (6.3)	–
Type III cryoglobulinemia, n (%)	7 (30.4)	1 (14.3)	6 (47.5)	0.366
Cryocrit (n,%)				
<1.0%	7 (30.4)	1 (14.3)	6 (37.5)	0.366
1.0–4.9%	10 (43.5)	3 (42.9)	7 (43.8)	1.000
5.0–19.9%	5 (21.7)	2 (28.6)	3 (18.8)	0.621
≥20.0%	1 (4.3)	1 (14.3)	0 (0.0)	–
Cryoglobulin level mg/L, median (range)	182.7 (16.4–10,925.0)	1000.9 (62.7–10,925.0)	101.4 (16.4–8012.2)	0.219

CGs = cryoglobulins; GI = gastrointestinal; Pos = positive.

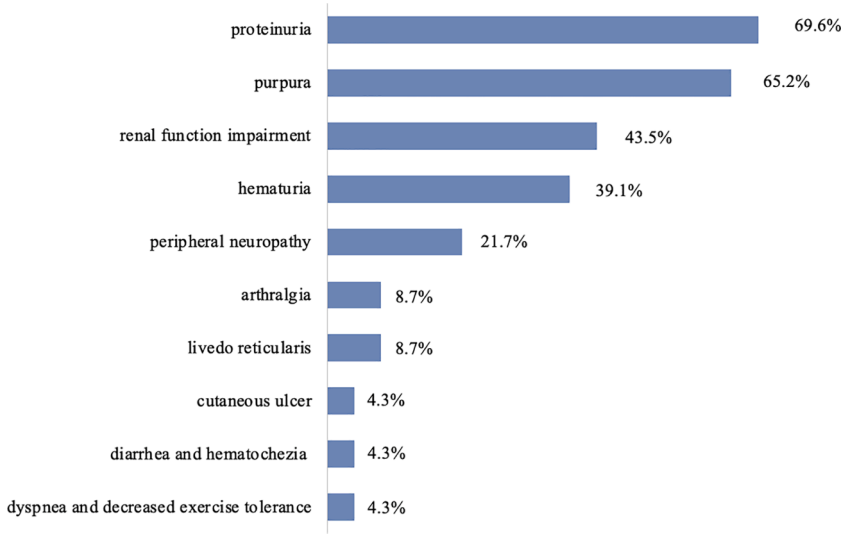

Fig. 1. The frequency and distribution of symptoms of cryoglobulinemia in all patients (n = 23) with HBV-related cryoglobulinemia.

(range, 14.29–76.34 ml/min/1.73 m^2), respectively.

The median concentration of cryoglobulins for patients with HBV-related cryoglobulinemia was 182.7 mg/L (range, 16.4–10,925.0 mg/L). Out of 20 patients, 14 patients exhibited a decrease in C3 serum levels, and the median concentration was 0.575 g/L (range, 0.251–0.726 g/L), and 13 patients had a decrease in C4 serum levels, with a median concentration of 0.026 g/L (range, 0.001–0.060 g/L). Additionally, 19 patients had RF titers, and 18 patients had elevated titers with a median level of 260.9 IU/ml (range, 20.8–11,404.9 IU/ml). Out of 21 patients, serum IFE was positive in 8 patients, including 7 with IgMκ as well as 1 with IgAλ, and urine IFE was positive in 2 patients out of 13 evaluable patients, both of whom showed the light chain of κ. Additionally, out of 16 patients with evaluable SPE results, 8 patients showed the existence of M protein, and the median M protein level was 3.89 g/L (range, 0.40–10.60 g/L).

Kidney biopsy was performed in 9 out of 16 patients with kidney involvement, and the results confirmed membranoproliferative glomerulonephritis (MPGN) in 6 patients and endocapillary proliferative glomerulonephritis in 3 patients. However, we observed HBsAg and hepatitis B core antigen (HBcAg) deposits in the kidneys in only two patients. Furthermore, one patient (patient 6) with GI tract involvement showed diarrhea and hematochezia accompanied by diffuse swelling of the gastric mucosa with multiple sites of erythema, as well as a thin and friable intestinal wall as revealed by gastrointestinal endoscopy. Additionally, another patient (patient 3) with cardiac involvement showed cardiomyopathy with an ejection fraction of 43% by echocardiography.

Seven patients showed positivity for HBsAg in cryoglobulins. Interestingly, there were two patients, one (patient 4) diagnosed with diffuse large B-cell lymphoma (DLBCL) and primary Sjogren's syndrome (pSS), and the other (patient 2) diagnosed with MALT lymphoma,

demonstrating the positivity of HBsAg in cryoglobulins, which confirmed that HBV infection was the underlying disease of cryoglobulinemia.

3.3. Treatment and outcomes

The detailed treatment and outcomes of all patients with HBV-related cryoglobulinemia are illustrated in the flow diagram (Fig. 2) and Table 1.

3.3.1. Antiviral therapy with NAs alone

Eight (34.8%) patients received ETV (0.5 mg/d) alone as first-line treatment, all of whom had mild-to-moderate symptoms, including purpura ($n = 5$), proteinuria ($n = 3$), arthralgia ($n = 1$), and peripheral neuropathy ($n = 1$). All patients achieved clinical remission, including 4 patients with complete remission (CR) and 4 patients with partial remission (PR). Of the 3 patients with laboratory response data available, 1 (patient 14) reached CR, and 2 (patient 5 and 10) reached PR. All patients treated with NAs alone exhibited HBV-DNA replication at baseline, and HBV-DNA replication was undetectable in 6 out of 8 patients, while the remaining 2 patients (patient 17 and 21) had a significant decrease in HBV-DNA copies. One patient (patient 10) relapsed because she stopped taking ETV without clinician permission; her relapse was accompanied by the return of her clinical symptoms, cryocrit elevation, and HBV-DNA replication. However, she reached PR in terms of both clinical remission and laboratory responses, and HBV-DNA replication was undetectable after she again received treatment with ETV alone.

3.3.2. Corticosteroid-based treatment

Twelve (52.2%) patients received NAs (ETV, 0.5 mg/d; LMV, 100 mg/d for patient 11) and corticosteroid (0.5–1 mg/kg)-based regimens as first-line treatment, containing NAs and corticosteroids ($n = 6$); NAs, corticosteroids, and cyclophosphamide (CTX) ($n = 2$); NAs, corticosteroids, and mycophenolate mofetil ($n = 2$); NAs, corticosteroids and plasma exchange (PE) ($n = 1$), and NAs, corticosteroids, and CTX in combination with PE ($n = 1$). The symptoms of patients treated with corticosteroid-based regimens consisted of proteinuria ($n = 11$) and renal function impairment ($n = 8$), including RPGN ($n = 1$); hematuria ($n = 7$), purpura ($n = 7$), livedo reticularis ($n = 2$), peripheral neuropathy ($n = 2$), arthralgia ($n = 1$), and life-threatening cardiac involvement ($n = 1$). One patient (patient 7) was lost to follow-up, and two patients (patient 11 and 12) died due to infection. Of the remaining 9 patients assessed for clinical remission, 3 patients reached CR, and 6 patients achieved PR. Of the 5 patients assessed for laboratory response, 2 patients had CR and 3 had PR. Furthermore, after treatment, HBV-DNA replication was undetectable in all of the patients ($n = 5$) with HBV-DNA replication at baseline.

3.3.3. Rituximab-based treatment

Three (13.0%) patients received a rituximab-based regimen as first-line treatment. One patient (patient 4) with severe peripheral neuropathy received ETV and rituximab (375 mg/m2, once). Another patient (patient 2) received ETV in association with rituximab (375 mg/m2), cyclophosphamide, and prednisone for 10 cycles, followed by rituximab and prednisone for 2 cycles due to renal function impairment (sCr 247 μmol/L at baseline). Both patients reached complete clinical remission and PR in terms of laboratory response along with undetectable HBV-DNA replication. However, one patient (patient 6) was treated with ETV (0.5 mg/72 h), rituximab (375 mg/m^2, once) and dexamethasone in combination with PE because of life-threatening GI tract involvement, cutaneous ulcer, and RPGN. The patient had no response to the treatment and died of RPGN (cryoglobulinemia-related event). One patient (patient 1) with multiorgan involvement (skin, peripheral nerves, and kidneys) initially received corticosteroids, CTX, ETV and PE treatment, which was accompanied by partial clinical remission and CR in terms of laboratory response. However, she relapsed after 32 months; received ETV, corticosteroids, and rituximab (200 mg, once a week for 3 weeks); and achieved PR in terms of clinical remission without HBV-DNA replication. However, she died due to COVID-19.

The median duration of follow-up was 44 months (range, 1–94 months). Of the 23 patients included in the present study, one patient was lost to follow-up, and 4 patients died during follow-up: one died due to COVID-19, one died due to cryoglobulinemia-related event, and two died due to therapy-related infection. Three patients experienced cryoglobulinemia progression. The three-year OS and PFS for patients with HBV-related cryoglobulinemia were 87.0% and 80.3%, respectively (Fig. 3).

4. Discussion

HBV infection is a less common etiology of cryoglobulinemia than

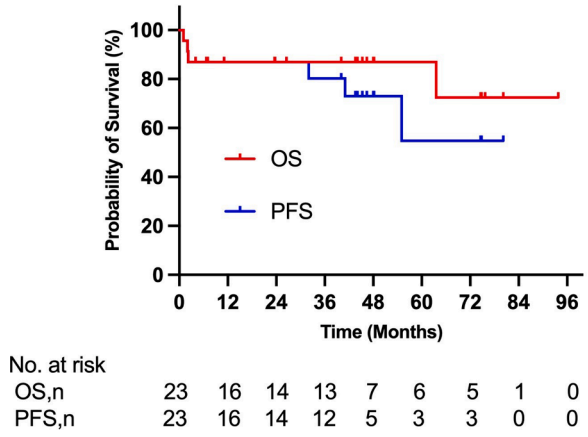

Fig. 3. Overall survival (OS) and progression-free survival (PFS) of all patients with HBV-related cryoglobulinemia ($n = 23$).

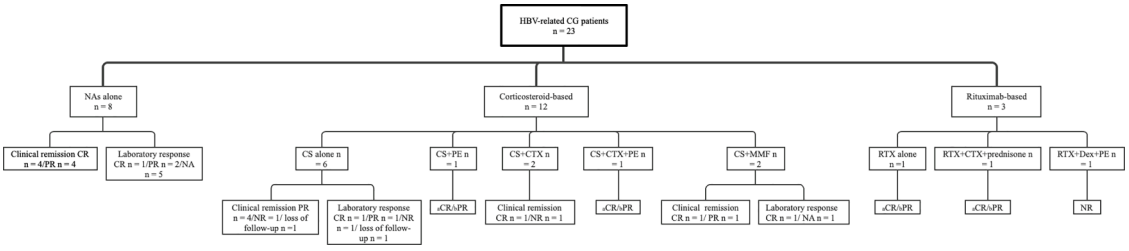

Fig. 2. Treatment and outcomes of all patients with HBV-related cryoglobulinemia.
CG = cryoglobulinemia; CR = complete response; CS = corticosteroids; CTX = cyclophosphamide; Dex = dexamethasone; MMF = mycophenolate mofetil; NA = not available; NAs = nucleot (s)ide analogs; NR = no response; PE = plasma exchange; PR = partial response; RTX = rituximab;
$_a$clinical remission; $_b$laboratory response; *All patients received antiviral therapy with NAs.

HCV infection (Ramos-Casals et al., 2012), with cryoglobulinemia rarely being reported in patients with HBV infection. To our knowledge, our study is one of the largest reported series of patients with HBV-related cryoglobulinemia thus far. This study describes the spectrum of clinical presentation and virological features and focuses on the treatments and outcomes of 23 patients with HBV-related cryoglobulinemia.

In the present study, we found that 4.6% of cases of cryoglobulinemia in our cohort were secondary to HBV infection, which was higher than the percentages of 4.5% and 1.8% reported by Mazzaro et al. (Mazzaro et al., 2018) and Ferri et al. (Ferri et al., 2004), respectively. This difference may be derived from the larger population with HBV infection in China. HBV infection was usually associated with mixed cryoglobulinemia and less commonly with type I cryoglobulinemia (Mazzaro et al., 2021), which was in accordance with our findings. Consistent with HCV-related cryoglobulinemia, in our cohort of HBV-related cryoglobulinemia patients, we found that the kidneys were the most commonly involved organs, with affected patients mainly presenting with proteinuria and kidney function impairment, followed by the skin, with affected patients mainly showing purpura. We also found life-threatening symptoms, including RPGN in two patients, GI tract involvement in one patient and cardiac involvement in one patient. However, the percentage of kidney involvement was higher than other western cohorts. (Boglione et al., 2013; Mazzaro et al., 2016) This discrepancy may derive from the limited sample size and the patient selection bias due to that many patients with hepatitis B adimitted to the nephrology department due to proteinuria, hematuria, or renal impairment, and then were diagnosed with cryoglobulinemia in our center. MPGN is the classic pathology in cryoglobulinemic glomerulonephritis. (Fogo et al., 2016, 2017) Nevertheless, our findings demonstrated that endocapillary proliferative glomerulonephritis was another typical pathology of patients with HBV-related cryoglobulinemia. Interestingly, HBsAg and HBcAg staining were positive in only two patients, which was similar to a previous study (Li et al., 2017) that observed HBsAg and/or HBcAg/hepatitis Be antigen (HBeAg) deposits in 3 out of 12 patients. The reason for the negativity of HBV antigen in the glomeruli of most patients with HBV-related cryoglobulinemia was unclear. It is possible that the proliferation and activation of monoclonal B cells play a more important role in the pathogenesis (Visentini et al., 2016) of kidney involvement than circulating immune complex-mediated inflammation (especially HBsAg).

In the present study, we found that 65.2% of HBV-related cryoglobulinemia patients were HBV-DNA positive, which was lower than other western cohorts (Boglione et al., 2013; Mazzaro et al., 2016). The difference may due to the fact that screening for hepatitis B was routinely performed in China, then many patients with HBsAg positive alone were found. Many studies (Galli et al., 2017; Mazzaro et al., 2016; Terrier et al., 2015) suggested that the diagnosis of HBV-related cryoglobulinemia should require the presence of HBV-DNA replication or serological HBsAg positivity. However, only positivity for HBsAg in cryoglobulins can confirm the diagnosis of cryoglobulinemia. We tested cryoglobulins for HBsAg presence in 7 out of 23 patients, and they all showed positivity for HBsAg in cryoglobulins. Furthermore, we compared the baseline clinical characteristics and laboratory findings between patients with HBsAg positivity in cryoglobulins and those without the evaluation of HBsAg in cryoglobulins. There were no significant differences between the two groups, which demonstrated that patients enrolled in our cohort conformed to the diagnosis of HBV-related cryoglobulinemia. Mixed cryoglobulinemia is usually associated with infections, CTDs and B-cell lymphoproliferative disorders (Desbois et al., 2019). Hence, it was difficult to distinguish the primary disease for cryoglobulinemia patients with various comorbidities. There were two patients in our cohort, one also diagnosed with DLBCL as well as pSS, and the other with MALT lymphoma, for whom the diagnosis of HBV-related cryoglobulinemia was confirmed by evaluation for HBsAg in cryoglobulins. Therefore, we considered that the examination for HBsAg in cryoglobulins was essential for cryoglobulinemia patients with HBV infection, which could confirm the diagnosis and distinguish the correct underlying disease for patients with comorbidities, which could also be conducive to precision-medicine-based treatment.

Due to its rarity, data on the treatment of HBV-related cryoglobulinemia are still scarce. The main goal of treatment is the early suppression of HBV replication to prevent organ complications and the appearance of lymphoproliferative disorders. (Mazzaro et al., 2019) Similar to HCV-related cryoglobulinemia, the treatment for HBV-related cryoglobulinemia includes antiviral therapy, immunosuppressive therapy and B-cell depleting therapy. (Muchtar et al., 2017) Antiviral therapy with NAs was the basis of treatment, and prior case series and studies (Boglione et al., 2013; Cakir et al., 2006; Mazzaro et al., 2016) have demonstrated a strong response to antiviral therapy. For patients with mild-to-moderate symptoms, treatment with antiviral therapy with NAs alone tended to be preferred. (Mazzaro et al., 2023, 2019) In our cohort, all patients ($n = 8$) treated with NAs monotherapy had significantly improved clinical symptoms and viral suppression. Furthermore, one patient relapsed because of unauthorised withdrawal of antiviral therapy with NAs, which led to the return of her clinical symptoms, viremia and increased cryoglobulins. However, the patient reached remission after receiving NA treatment alone again. Unlike HCV-related cryoglobulinemia, it was necessary for patients with HBV-related cryoglobulinemia to continue antiviral treatment even if the symptoms associated with cryoglobulinemia disappeared.

For severe and relapsed patients, immunosuppressive agents and rituximab-based regimens could be considered based on adequate antiviral therapy and close monitoring (Mazzaro et al., 2021; Muchtar et al., 2017; Quartuccio et al., 2023). A previous study (Mazzaro et al., 2016) summarized the cases of 5 patients treated with NAs and corticosteroids, and 3 patients obtained clinical reemission. In our cohort, 9 out of 12 patients treated with corticosteroid-based regimens achieved clinical remission along with undetectable HBV-DNA replication. Due to the high risk for the reactivation of HBV (Mozessohn et al., 2015), there is a lack of data about the use of rituximab-based regimens for HBV-related cryoglobulinemia. The Italian Study Group of Cryoglobulinemia (GISC) recommended that rituximab should be used cautiously and HBV-DNA or HBsAg titers should be monitored. (Quartuccio et al., 2023) According to our data, 2 out of 3 patients received antiviral therapy, and rituximab-based regimens led to clinical remission and a reduction in cryoglobulins without HBV reactivation.

The major limitation of our current study was that it was a single-center retrospective study, which might limit the representativeness and generalizability of our results. Another limitation was that we did not conduct testing for HBsAg in cryoglobulins for all patients, which might lead to an unclear diagnosis of HBV-related cryoglobulinemia. However, in our cohort, we did not find a remarkable difference between patients with HBsAg positivity in cryoglobulinemia and those for whom HBsAg in cryoglobulins was not evaluated.

In summary, HBV-related cryoglobulinemia is uncommon. The evaluation of HBsAg in cryoglobulins can confirm the diagnosis. Antiviral therapy with NAs is the core of treatment. Corticosteroids and rituximab-based regimens could be given to patients with severe clinical symptoms along with adequate antiviral therapy, and patients need to be closely monitored for therapy-related infection.

Author contributions

Cao XX contributed to study conception and design; Han HX led the data management and statistical analyses and draft the manuscript. Su W contributed to cryoglobulin detection. Li J and Zhou DB contributed to patient enrollment. All authors reviewed and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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