Cryoglobulinemic vasculitis and glomerulonephritis: concerns in clinical practice

Yi-Pu Chen, Hong Cheng, Hong-Liang Rui, Hong-Rui Dong

Division of Nephrology, Beijing Anzhen Hospital, Capital Medical University, Beijing 100029, China.

Abstract

Objective: Cryoglobulinemia often causes systemic vasculitis, thereby damaging to skin and internal organs including kidneys, even life-threatening. This review aimed to introduce the advances in understanding, detection, and treatment of this disease in recent years, with a particular concern to clinical practice.

Data sources: All the data in this review were from the English or Chinese literature in the PubMed and China National Knowledge Infrastructure databases as of March 2019.

Study selection: This review selected important original articles, meaningful reviews, and some reports on cryoglobulinemia published in recent years and in history, as well as the guidelines for treatment of underlying diseases which lead to cryoglobulinemia.

Results: Diagnosis of cryoglobulinemia relies on serum cryoglobulin test, in which to ensure that the blood sample temperature is not less than 37°C in the entire pre-analysis phase is the key to avoid false negative results. Cryoglobulinemic vasculitis (Cryo Vas), including cryoglobulinemic glomerulonephritis (Cryo GN), usually occurs in types II and III mixed cryoglobulinemia, and can also be seen in type I cryoglobulinemia caused by monoclonal IgG3 or IgG1. Skin purpura, positive serum rheumatoid factor, and decreased serum levels of C4 and C3 are important clues for prompting types II and III Cryo Vas. Renal biopsy is an important means for diagnosis of Cryo GN, while membranous proliferative GN is the most common pathological type of Cryo GN. In recent years, great advances have been made in the treatment of Cryo Vas and its underlying diseases, and this review has briefly introduced these advances.

Conclusions: Laboratory examinations of serum cryoglobulins urgently need standardization. The recent advances in the diagnosis and treatment of Cryo Vas and GN need to be popularized among the clinicians in related disciplines.

Keywords: Cryoglobulin; Vasculitis; Glomerulonephritis; Monoclonal immunoglobulin; Hepatitis C virus; Autoimmune disease

Introduction

Cryoglobulins (CGs), which was first named by Lerner and Watson in 1947,[1] are immunoglobulins that precipitate or form a gel when exposed to temperatures below 37°C and re-solubilize when re-warmed. Cryoglobulinemia refers to the presence of CGs in serum (positive results in qualitative tests and/or >0.05 g/L concentration in quantitative tests determined by cryocrit). Cryoglobulinemia may not have any clinical symptom, but can also cause a wide spectrum of clinical presentations including skin lesions, arthralgia, peripheral neuropathy, single, or multiple organ damage. The symptomatic cryoglobulinemia is called cryoglobulinemic disease or cryoglobulinemic vasculitis (Cryo Vas).[1-6] Kidney is one of the most easily involved organs in Cryo Vas and cryoglobulinemic glomerulonephritis (Cryo GN) was reported as early as in 1966.[7-8]

In 1974, Brouet *et al*[9] proposed a classification of CGs based on the clonality and type of immunoglobulins, which has been used until now because of its good correlation with clinical practice. CGs are categorized into the following three types: Type I CGs consist of a single monoclonal immunoglobulin (mostly monoclonal IgG or IgM and rarely monoclonal IgA), which accounts for 10% to 15% of cases; type II CGs are composed of monoclonal IgM (IgM κ in more than 90% of cases) with rheumatoid factor (RF) activity and polyclonal IgG, which accounts for 50% to 60% of cases; type III CGs are constituted by polyclonal IgM with RF activity and polyclonal IgG, which accounts for 25% to 30% of cases. Types II and III are also referred to as mixed CGs, because they consist of two types of immunoglobulins.[2-6,9-11] Actually, type III is a transitional form that can evolve to type II during the progressive process of cryoglobulinemia. In a few patients,

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Correspondence to: Prof. Yi-Pu Chen, Division of Nephrology, Beijing Anzhen Hospital, Capital Medical University, Anzhen Road No. 2, Chaoyang District, Beijing 100029, China
E-Mail: chen_yipu@163.com

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it has been detected that oligoclonal IgM or mixed polyclonal and monoclonal IgM combined together with polyclonal IgG, which is named as type II-III CGs and considered as an intermediate state of the transition from type III to type II.[10-13] In addition, it was occasionally reported that mixed CGs were not composed of IgM-IgG, but of other immunoglobulin combinations, such as IgG-IgG, IgA-IgG, or IgM-IgG-IgA.[14,15]

Type I cryoglobulinemia is caused by B lymphocyte/lymphoplasmacyte proliferative diseases including hematological malignancies and monoclonal gammopathy of unknown significance (MGUS). The former mainly includes multiple myeloma (MM), Waldenström macroglobulinemia (WM), chronic lymphocytic leukemia and lymphoma. In two larger series of studies on type I cryoglobulinemia, MGUS accounted for 43.8% (28/64 cases) and 40.6% (26/64 cases) of total cases, respectively.[3,16] Type II and type III mixed cryoglobulinemia (MC) is often caused by infections or autoimmune diseases. Infections caused by a variety of pathogens, such as viruses, bacteria, fungi, protozoa, and parasites may induce MC.[2,11,15] However, among them, hepatitis C virus (HCV) infection is the most important causal factor. The incidence of HCV infection in patients with MC varies among different geographical regions, generally between 70% and 90%, and in the Mediterranean basin over 90%.[2,13,14,17,18] However, in China the incidence of hepatitis B virus (HBV) infection in cryoglobulinemia seems to be equal to or more than HCV infection. The information from three larger nephrological centers in 2014 to 2018 is as follows: in 30 cases of cryoglobulinemia with or without Cryo GN the incidence of HBV and HCV infection was equal (both 36.7%)[19]; in 40 cases of cryoglobulinemia with Cryo GN the incidence of HBV infection was higher than HCV infection (30% *vs*. 10%)[20]; in our 28 cases of MC with Cryo GN the incidence of HBV infection was also higher than HCV infection (21.4% *vs*. 10.7%).[21] Many kinds of autoimmune diseases may cause MC, but among them, Sjögren's syndrome, systemic lupus erythematosus, and rheumatoid arthritis are most common causal factors.[2,10,15,21,22] Besides infections or autoimmune diseases, some researchers consider B-cell non-Hodgkin lymphoma is also one of the causes of MC.[2] However, it has been known that HCV infection-driven B-cell activation and proliferation can lead to both MC and lymphoma.[23] Therefore, when these two diseases are both associated with chronic HCV infection, it must be carefully judged whether the relationship between them is accompanying (both secondary to HCV infection) or causal (MC caused by lymphoma).

This article will review the recent progress in the diagnosis and treatment of Cryo Vas and GN, especially the problems concerned in clinical practice.

Clinical and Laboratory Findings: Prompt Clues for Cryoglobulinemia

There are two principal mechanisms by which CGs cause disease manifestations. The first mechanism is related with blood stasis due to hyperviscosity and occlusion of small and medium blood vessels due to CG precipitation. Such patients frequently appear hyperviscosity syndrome (headache, dizziness, blurry vision, hearing loss, and epistaxis, etc), livedo reticularis, Raynaud phenomena, acrocyanosis, cutaneous necrosis, and ulcers in the distal region of body (hands, feet, lips, ears, and nose), which often occur or worsen during cold exposure. The above manifestations are common in type I cryoglobulinemia, especially with high concentrations of CGs, but less in MC.[2,3,5,6,16]

The second mechanism is related with immune-mediated vasculitis of small and medium blood vessel, which is common in types II and III MC, while less in type I cryoglobulinemia.[2,6,21] In MC IgM with RF activity (as autoantibody) binds IgG (as antigen) to form immune complex, which deposits on vascular wall and then activates complement system through classical pathway, leading to occurrence of vasculitis.[2,6,21] In addition, it has been known that monoclonal IgG3 in type I cryoglobulinemia has unique property to spontaneously self-aggregate by non-specific Fc-Fc interaction and then deposit on vascular wall. The self-aggregated monoclonal IgG3 is able to bind C1q via the CH2 domain on its heavy chain, and consequently to activate the complement system, leading to vasculitis.[11,21,24-27] In addition, monoclonal IgG1 may also have the ability to bind C1q via the CH2 domain and then activate complement system.[21,27] Although the C1q-binding activity of monoclonal IgG1 is weaker than IgG3, it has been reported that monoclonal IgG1 in type 1 cryoglobulinemia can activate complement system by classical pathway, leading to Cryo GN.[21,28-30]

The main clinical manifestations of immune-mediated vasculitis are as follows: skin purpura (40%–98%) which usually is the first sign; arthralgia (20%–90%); peripheral neuropathy (20%–80%); renal involvement (20%–50%). In addition, other organs (such as gastrointestinal tract, liver, lung, heart, and central nervous system) can also be involved to a lesser extent.[5,11,13-15,31] The important laboratory findings include positive serum RF (45%–95%), low level of serum C4 (65%–100%), and C3 (20%–70%) in MC.[5,14,21,29] In the type I cryoglobulinemia which is composed of monoclonal IgG3 or IgG1, low levels of serum C4 and C3 may be also observed.[21,29,30]

Based on the above descriptions, the following clinical and laboratory features are important prompt clues for cryoglobulinemia: (1) when a patient with chronic infection or autoimmune disease develops positive serum RF, decreased serum C4 and C3 levels, especially accompanied with skin purpura, MC should be highly suspected; (2) when a patient with monoclonal gammaglobulinemia develops cutaneous damages, for example, cutaneous necrosis and ulcers, which often occur or worsen during cold exposure, type I cryoglobulinemia should be suspected; (3) when lower serum C4 and C3 levels appear in a patient with IgG3 or IgG1 monoclonal gammaglobulinemia, especially accompanied with skin purpura and/or GN, type I cryoglobulinemia should also be suspected. Once the above clues appear, the serum CGs should be tested immediately.

Detection of Serum CGs: Basis for Diagnosis of Cryoglobulinemia

So far there is no internationally accepted standard for serum CG detection. Generally, this detection includes three phases, that is, pre-analysis phase, analysis phase, and post-analysis phase (analysis and summary of experimental results). Here, we will discuss in detail the experimental steps and precautions of the first two phases.

Pre-analysis phase: blood sample collection, transport, blood clotting, and serum separation

The correct operation steps are as follows: blood sample (about 20 mL) is collected into tubes (without anti-coagulant or serum separating gel) pre-warmed at 37°C; tubes are placed in a warm water or sand container (38–40°C) to transport to laboratory; blood is allowed to clot in a 37°C incubator for at least 1 h; serum is separated from the clot by centrifugation at 37°C and then used for analysis. It is extremely important to ensure that the temperature never fall below 37°C at any time of the pre-analysis phase. Failure to meet this requirement is the most common cause leading to false negative results.[32-36] In 2008 Vermeersch *et al*[37] published an investigation result, which was conducted by United Kingdom National External Quality Assurance Scheme (UKNEQAS). They surveyed the practice of serum CG detection in 137 laboratories in Europe and found that only 36% of laboratories met the above temperature requirements in the pre-analysis phase.[34,37] In China we do not have such survey data, but according to our observations, it will not be better than above situation. So, the non-standard operation in the pre-analysis phase and the false negative results caused by it are very noteworthy problems in clinical work.

Analysis phase: qualitative test, quantitative test, immunotyping test, and etiological analysis

Qualitative test

Serum is incubated at 4°C for seven days and inspected for precipitates every day; if precipitates appear, the sample should be warmed to 37°C for 2 to 3 h on the seventh day to observe whether the precipitates re-solubilize. Only when precipitates appear at 4°C and re-solubilize at 37°C, can the result be judged to be positive [Figure 1A]. On the contrary, no precipitates appear at 4°C or the precipitates cannot re-solubilize after rewarming, the result should be judged as negative.[33-38] When it is hard to be sure if there is precipitate, centrifuging the suspect sample and then looking for a pellet in the tube bottom should be recommended, which can improve the accuracy of judgment.[39] The survey by UKNEQAS found that 30% laboratories allowed serum incubation at 4°C for less than 3 days, and 19% of laboratories did not re-solubilize the cryoprecipitates at 37°C. The above non-standard operations may result in false negative and false positive results, respectively.[34,37] Type I CGs usually exist as a high blood concentration (>5 g/L) that can begin to precipitate within a few hours, but type III CGs usually have a low blood concentration (<1 g/L) that may take several days (up to 7 days) to precipitate, so insufficient incubation time at 4°C may miss the diagnosis of type III CGs, leading to false negative results.[33-39] In addition, to avoid false positive results, it is necessary to check if the cryoprecipitates can re-solubilize at 37°C. It has been reported that the contaminants of red blood cell debris or fibrin may also precipitate during the process of storage in refrigerator at 4°C, but cannot be re-solubilized at 37°C.[36,38] Finally, if the blood sample is taken from a patient receiving heparin therapy, for example undergoing hemodialysis or plasmapheresis, two kinds of cryoprecipitates, that is, cryofibrinogens and heparin-fibronectin complexes, may be produced in the sample containing heparin. Their physical characters are quite similar to those of CGs. In this case, only the immunochemical analysis can identify the nature of the cryoprecipitates, determining whether it is cryoglobulin.[33,34,38]

Quantitative tests

There are three ways to quantify CGs. (1) Cryocrit: serum in Wintrobe tube is incubated at 4°C for 7 days, then centrifuged at 4°C, and finally the percentage of cryoprecipitate volume to total serum volume is determined by

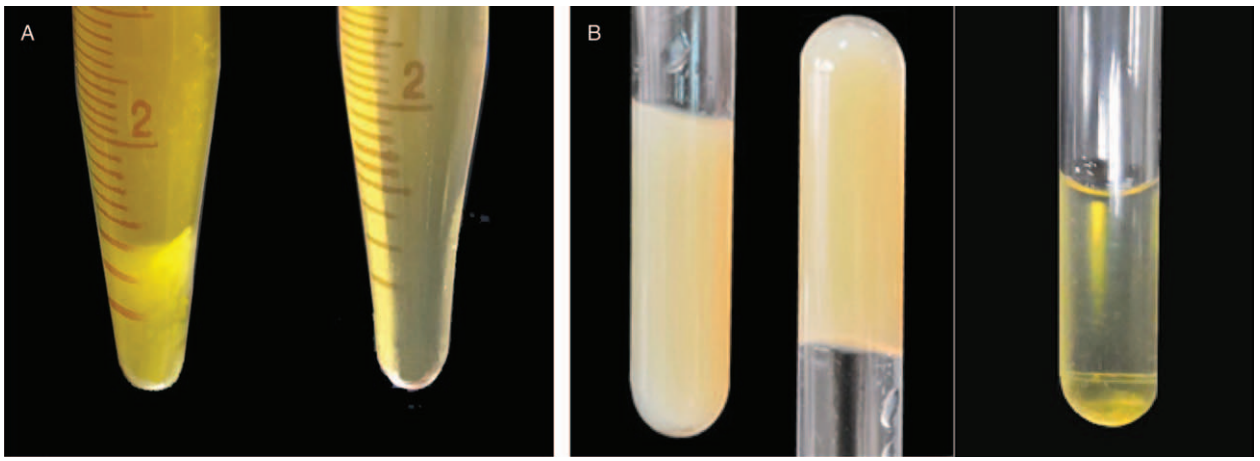

Figure 1: The cryoprecipitates separated from serum after storage in a refrigerator at 4°C for 7 days. (A) Flocculent precipitates at 4°C (left); dissolution of precipitates after re-warming at 37°C (right). (B) Gelatinous precipitates at 4°C (left); dissolution of precipitates after rewarming at 37°C (right).

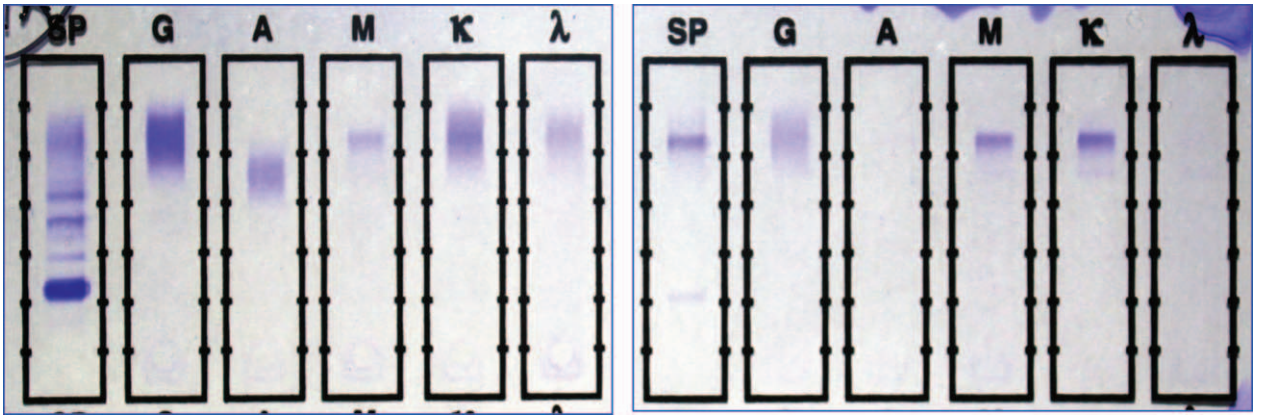

Figure 2: IFE of serum and heating-resolubilized cryoglobulins in a patient with type II mixed cryoglobulinemia. Left image: Serum IFE showed monoclonal IgM and light chain κ bands and polyclonal IgG, IgA, and light chain λ bands. Right image: Cryoglobulin IFE showed monoclonal IgM and light chain κ bands and polyclonal IgG band. The monoclonal bands in cryoglobulin sample are much clearer than those in serum sample. IFE: Immunofixation electrophoresis.

observing the scale mark on tube wall. This evaluation method is convenient and inexpensive, but it is quite inaccurate and insensitive.[33-38] (2) Total protein content: cryoprecipitates are washed with cold saline solution at 4°C at least three times to remove serum. After washing, cryoprecipitates are re-suspended in saline solution which volume is the same as the volume of supernatant discarded, and then re-solubilized by rewarming to 37°C overnight. The re-solubilized cryoprecipitates will be used for the quantitative tests of total proteins or immunoglobulins and for immunotyping tests.[33-38] It has been reported that many protein quantification methods are available including Bradford assay, Folin assay, and optical densitometer detection (280 nm).[33,38] (3) Immunoglobulin concentration: besides abundance of CGs, there are some other proteins such as albumin, fibrinogen, fibronectin, complement components, viruses, and bacteria in cryoprecipitates.[33,34,37-39] The concentration of "CGs" obtained by cryocrit estimation or total protein quantification actually contains not only CGs but also aforementioned other proteins. In order to overcome this problem, immunoglobulins in cryoprecipitates are quantified by immunonephelometry or as the area under the curve in the gamma region following electrophoresis.[33,37,38] The survey by UKNEQAS found only 39% laboratories conducted the above quantitative tests.[37] So, this state urgently needs improvement. Finally, when interpreting the results of quantitative tests, it should be noted that serum CG concentrations are not always related to the severity of Cryo Vas.[32-34,37,38] However, changes in serum CG concentration during treatment can still reflect the therapeutic effect to a certain extent.[18,34]

Immunotyping tests

Once the diagnosis of cryoglobulinemia is established, immunotyping tests should be performed immediately because the therapeutic regimens need to be created on the typing basis. It has been reported that the following tests are available for CG typing: protein electrophoresis, immunoelectrophoresis, immunosubtraction by capillary electrophoresis, two-dimensional polyacrylamide gel electrophoresis, immunoblotting and immunofixation electrophoresis.[9,10,33-39,40] Type I and type II cryoglobulinemia can detect monoclonal immunoglobulin, while type III does not. Immunofixation electrophoresis is now used in most laboratories, because its operation is not complicated while its results are quite reliable, which has been considered by some researchers as the "gold standard" for CG immunotyping.[33] In our experience, immunofixation electrophoresis carried about using heating-resolubilized CGs may be easier to recognize monoclonal immunoglobulin band(s) than serum immunofixation electrophoresis, because other non-CG components in serum have been removed [Figures 2 and 3].

Etiological analysis

If necessary, heating-resolubilized CGs can also be used for etiological examination. For autoimmune diseases such as systemic lupus erythematosus and Sjogren's syndrome, their corresponding autoantibodies can be detected; for infectious diseases such as HCV infection, the anti-HCV antibody, and HCV RNA can be tested.[17,36]

Before ending this paragraph, two points need to be emphasized. First, the cryoprecipitates observed by the naked eye are usually flocculent, but sometimes gelatinous[6,10,33,34,38] [Figure 1B]. According to our experience, when the cryoprecipitates are gelatinous, the gel may contain more serum components which cannot be cleared away by washing. Therefore, it cannot be used for further research, that is, quantification, immunotyping, and etiological analysis of CGs. Second, when the clinical manifestations highly suggest cryoglobulinemia but the serum CGs test is negative, it needs to be repeated the test 2 or 3 times by using fresh blood sample in strict accordance with the operating protocol.[6,34] Moreover, it may be occasionally observed that a patient has overt clinical manifestations of Cryo Vas, but repeat serum CG tests all are negative. Ferri *et al*,[18] as well as Roccatello *et al*,[10] explain that this sometimes occurs transiently in the natural course of the disease, which is resulted from that the amount of CGs in the circulation changes greatly at different points in time. In this situation, it is necessary to do a long term follow up and regular serum CGs tests.

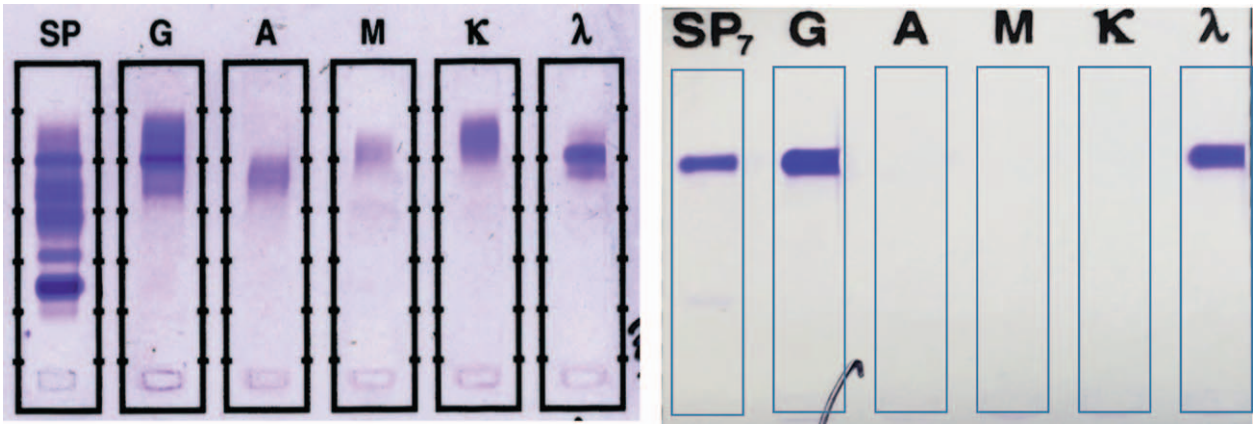

Figure 3: IFE of serum and heating-resolubilized cryoglobulins in a patient with type I cryoglobulinemia.[21] Left image: Serum IFE showed monoclonal IgG and light chain λ bands and polyclonal IgM, IgA, and light chain κ bands. Right image: Cryoglobulin IFE showed monoclonal IgG and light chain λ bands which are much clearer than those in serum sample. IFE: Immunofixation electrophoresis.

Glomerulonephritis: Renal Involvement of Cryoglobulinemic Vasculitis

Cryo Vas frequently involves internal organs including kidneys. GN is the principal manifestation of renal involvement.[2,4,5] As mentioned above, renal involvement is common in MC and less frequently in type I cryoglobulinemia.

Clinical presentations

The major clinical presentations include hematuria (almost 100% microscopic hematuria and occasional macroscopic hematuria), proteinuria (almost 100%), hypertension (35%–85%), and chronic renal insufficiency (40%–85% at the time of diagnosis). In addition, 20% to 50% of patients present with nephrotic syndrome and 20% to 30% of patients acute nephritic syndrome. These clinical manifestations are related with the pathological types of Cryo GN which are often proliferative GN, especially membrane proliferative GN (see below).[21,41-44] In a few series of patients acute kidney injury (AKI) was also reported, accounting for 10% to 17% of total cases.[2,21,43,45] According to our experience, the AKI is sometimes not caused by Cryo GN itself, but by its concomitant renal diseases, such as active lupus nephritis (LN) or myeloma cast nephropathy, or by therapeutic drugs (via toxic effect or allergic reaction). Therefore, in the case of AKI, its etiology should be carefully identified and renal biopsy should be carried out as necessary.

Pathological features

Membranoproliferative GN is the commonest pathological type in Cryo GN, observed in 70% to 90% of cases.[2,11,41-44] The rest are other types of proliferative GN, for example, endocapillary proliferative GN, mesangial proliferative GN, and rare crescentic GN.[21,42-47] By light microscopy, there are some characteristics which can help distinguish proliferative Cryo GN from primary proliferative GN. Eosinophilic and periodic acid-Schiff stain (PAS)-strongly positive deposits in the sub-endothelial sites and within capillary lumens (so-called pseudothrombi or hyaline thrombi) often appear in Cryo GN; diffuse intra-capillary infiltration of massive monocytes/macrophages (in acute and chronic stages) and less polymorphonuclear leukocytes (in acute stage) are often observed in Cryo GN.[21,28,42-44,48] By immune-fluorescence microscopy, there are granular immune deposits in mesangium and capillary wall, which often sketches a petal-like glomerular outline in the type of membranoproliferative GN. In types II and III mixed Cryo GN, the immune deposits contain IgM, IgG, C3, and C1q, and, besides these, in type II also contain monoclonal light chain κ or λ (the former accounts for more than 90% of cases, while the latter less than 10%); In type I Cryo GN caused by monoclonal IgG1 or IgG3, the immune deposits consist of IgG, IgG1 or IgG3, C3, C1q, and monoclonal κ or λ. The above components of immune deposits may also exist in pseudothrombi of capillary lumens.[21,24,28,41,48] By electron microscopy, the deposits in mesangial, sub-endothelial and capillary lumens are often seen. Deposits may show vague short fibrillary or granular sub-structure, and the deposits containing monoclonal CGs may show highly organized microtubular or annular sub-structure (at magnification ×20,000–50,000). However, not all electron microscopic specimens of patients can detect the above sub-structures, so that the diagnosis of Cryo GN cannot be denied simply because no sub-structures have been found.[21,41-44,48]

Finally, there is another issue in clinical practice that needs to be discussed here. Systemic lupus erythematosus, including LN, often complicated with MC, which incidence rate is 25% to 49%, and such patients easily develop glomerular pseudethrombi.[21,22,49] However, LN itself is also prone to glomerular thrombi, which may occur in 41% to 49% of patients.[50,51] So, it is very important how to differentiate the intra-glomerular thrombus-like deposits as real thrombi or pseudothrombi, when LN complicated with MC. According to our experience, immunofluorescence staining including dual immunofluorescence staining is very helpful for this identification. If thrombus-like deposit shows negative staining of fibrin and positive staining of immunoglobulin which component depends on the type of cryoglobulin, it is cryoglobulinemic pseudo-thrombus; while the contrary result supports real thrombus [Figure 4].

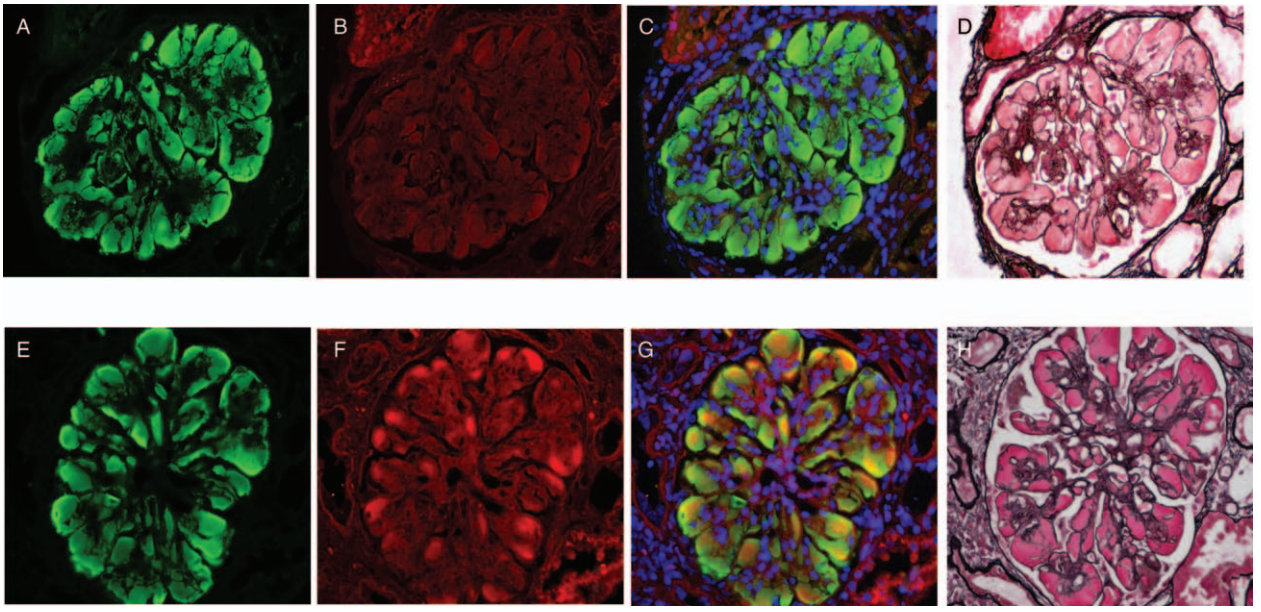

Figure 4: Dual immunofluorescence staining of kidney sections from a case of type III cryoglobulinemia. There were cryoglobulin pseudothrombi in the glomerular capillary lumens with a lot of sub-endothelial deposits. (A) Positive staining with isothiocyanate-labeled anti-IgM antbody; (B) negative staining with rhodamine-labeled anti-fibrin-related antibody; (C) merging image of (A) and (B); (D) staining with PASM and Masson trichrome. When anti-IgG antibody was used to replace anti-IgM antibody, the staining results were the same. (E) Positive staining with isothiocyanate-labeled anti-IgG antibody; (F) positive staining with rhodamine-labeled anti-IgM antibody; (C) merging image of (E) and (F); (G) staining with PASM and Masson trichrome. The original magnification of all images is ×400.

Treatment Regimens: for Cryo Vas and Glomerulonephritis

So far, evidence-based high-quality treatment for Cryo Vas and GN is limited, therefore most of the following treatment recommendations come from expert experience. The establishment of Cryo Vas and GN therapeutic regimens needs to be based on typing and etiological analysis of cryoglobulinemia. For different cryoglobulinemic types and different underlying disorders, the treatment measures are different. In the following, we are going to introduce the progress and current status of principle therapies, as well as the impacts of renal insufficiency on them.

Type I cryoglobulinemia

The therapeutic regimens for the underlying diseases of type I cryoglobulinemia can be divided into the following three categories.[52]

For diseases of clonal plasma cells

This group of diseases contains malignant MM and MGUS (when MGUS leads to kidney damage, including Cryo GN, its name will be changed to monoclonal gammopathy of renal significance, abbreviated as MGRS), which usually secret monoclonal IgG (or rare IgA). They should be treated with reference to the anti-MM regimens: (1) Proteasome inhibitors: bortezomib-based regimens are the cornerstone of treatment. Bortezomib has been often used in patients with severe renal insufficiency and even dialysis without adjusting the dose. However, "The Renal Drug Handbook" suggests that bortezomib dosage may require to be reduced and careful monitoring is needed when glomerular filtration rate (GFR) is less than 10 mL/min. Besides bortezomib new proteasome inhibitors such as carfilzomib and ixazomib has been approved by Food and Drug Administration (FDA) for the treatment of recurrent/refractory MM.[52-57] (2) Immunomodulatory drugs: thalidomide-based regimens are alternative options of treatment for type I Cryo Vas and GN. Thalidomide is not excreted via the kidneys and thus does not need dosage modification in renal insufficiency even renal failure. Lenalidomide is rarely used to treat type I Cryo GN patients with impaired renal function, because it is partially cleared by the kidneys. Moreover, it can sometimes worsen renal function and lead to AKI. Pomalidomide, a third-generation immune-modulatory drug, has not been reported to be used in the treatment of Cryo Vas and GN until now.[52-54,57,58] (3) High-dose melphalan with autologous peripheral blood stem cell transplantation: the regimen may be a therapeutic option for eligible patients with MM and is feasible in patients with renal failure and even requiring dialysis. However, melphalan dosage requires to be reduced when GFR is less than 50 mL/min.[52-54,57,59] (4) Corticosteroids and alkylating agents: previously the combination of high dose corticosteroid and cyclophosphamide was often used as a main treatment of type I Cryo Vas and GN, but now corticosteroid or cyclophosphamide, only as a component, are integrated into therapeutic regimens based on bortezomib or thalidomide.[3,16,60] Cyclophosphamide dosage needs to be reduced when GFR is less than 20 mL/min, while another alkylating agent, bendamustine, is not eliminated via kidneys, so it is likely to be a good alternative in the case of renal insufficiency.[16,52,53,57]

For diseases of clonal lymphoplasmacytic cells

This group of diseases contains malignant WM and MGUS, which usually secret a monoclonal IgM. Their

treatment should rely on anti-WM regimens, that is, rituximab-based regimens.[52,61] Within 48 h following rituximab infusion, a transient elevation of serum CG levels and even a transient disease exacerbation (so-called vasculitis flare) may happen, especially in the patients with high baseline levels of CGs. This is caused by massive CG released from the lymphoplasmacytic cells activated or killed by rituximab, which needs to be concerned. Reducing the high serum monoclonal IgM levels by pre-performed plasma exchange may effectively prevent the vasculitis flare.[3,16,54,62]

For diseases of malignant clonal B-lymphocytes

They are mainly chronic lymphocytic leukemia and B-cell non-Hodgkin lymphoma. Type I cryoglobulinemia is rarely caused by them. For treatment of these malignancies, rituximab-containing regimens are recommended.[63,64] When rituximab is used in combination with fludarabine, attention should be paid to the effects of renal insufficiency on fludarabine excretion. Approximately 60% of an administered dose of fludarabine is excreted in the urine within 24 h, so its dosage needs to be reduced when GFR is less than 70 mL/min, and it should be avoided to use in renal failure.[52,57]

In all cases, if the patient has severe symptomatic hyperviscosity, refractory cutaneous ulcers, or severe organ involvement, plasmapheresis including double-filtration plasmapheresis can be used as an adjunct therapy of chemotherapy.[52-54,60,65-67] When performing plasmapheresis, the replacement fluids for plasma exchange should be warmed to body temperature before infusion to avoid CG precipitation in circulation and blocking glomerular capillaries, leading to AKI.[54,68] According to our experience, when the blood concentration of CGs is quite high, if use double-filtration plasmapheresis, the secondary filter will be easily blocked, resulting in failure of plasmapheresis, so in this case double-filtration plasmapheresis might not be suitable for use. However, so far there are few evidences that plasmapheresis can improve the long-term outcome of diseases.[52-54,60,65-67]

Types II and III mixed cryoglobulinemia

General therapeutic principles

The underlying diseases which result in types II and III MC are mainly infections and autoimmune diseases. For infections, specific anti-pathogen therapy should be as first-line treatment, and effective anti-pathogen therapy may cause sustained remission of Cryo Vas. Corticosteroids and immunosuppressive agents, even rituximab, should only be used as second-line options. They are suitable in treatment of the patients with unrelieved Cryo Vas after infection elimination, or combined with anti-pathogen medicines in treatment of the patients with urgent/life-threatening Cryo Vas, for example, rapidly progressive Cryo GN and central nervous system, heart or pulmonary involvement. It is not suggested to use corticosteroids, immune-suppressive agents, and rituximab in the absence of anti-pathogen therapy, which might aggravate infections and increase mortality.[69] For autoimmune diseases, corticosteroids with or without immunosuppressive agents should be used as first-line treatment, and rituximab can be selectively used for refractory and/or severe cases. Effective immunosuppressive therapy can simultaneously alleviate autoimmune diseases and its MC complication.[31,54,70]

For the type II MC patients with clonal proliferation of plasma cells, is it needed to treat clonal proliferative plasma cells with protease inhibitors or immunomodulatory drugs, as done in type I cryoglobulinemia? To date it is unclear. In 2011, France researchers reported one case of parvovirus B19-induced type II MC, who received the treatment with prednisone, plasmapheresis, and rituximab, but Cryo Vas and GN did not improve. After that, the patient received lenadomide therapy and the Cryo Vas and GN obtained complete remission.[71] It is not clear whether the efficacy of lenalidomide comes from inhibiting the clonal proliferation of plasma cells or from stimulating anti-viral immunity (ie, enhancing the activity of virus-specific cytotoxic $CD8^+$ T cells).[31,71] We think that the treatment with the medicines that inhibit plasma cell clonal proliferation in selective patients with type II MC is worth of further exploration.

Anti-viral treatment regimens of HCV-related mixed cryoglobulinemia

HCV infection is the leading cause of types II and III MC. Anti-viral therapy is the key therapeutic approach. Sustained viral clearance can often improve or cure Cryo Vas. Anti-HCV therapeutic regimens have undergone a development process: (1) Combination therapy of pegylated interferon (Peg IFN) and ribavirin: prior to 2011, this regimen was the main therapeutic regimen for HCV infection, given 12 months. It only produced a lower sustained virological response (SVR, 20%–60%), but had a high relapse rate (about 60%) of HCV infection and Cryo Vas.[72-74] (2) Triple therapy: after the advent of the first generation of direct-acting anti-virals (DAAs), that is, NS3/4A protease inhibitors telaprevir and boceprevir in 2011, a triple therapeutic regimen combining Peg IFN, ribavirin and telaprevir or boceprevir was used for anti-HCV therapy in the patients with HCV genotype 1 infection. It improved SVR rates (65%–70%) in the patients with HCV-related Cryo Vas, but it needed a long therapeutic period of 48 weeks and its severe adverse events (SAEs) reached 47%.[72-74] Therefore, the therapeutic regimen was no longer recommended by the guidelines established by the European Association for the Study of the Liver in 2015 and by the American Association for the Study of Liver Diseases/Infectious Diseases Society of America in 2015,[74,75] as well as by the guideline of the World Health Organization in 2016.[76] (3) IFN-free, DAA-based combination therapy: in 2013, the second-generation of DAAs, that is, NS3/4A protease inhibitor simeprevir and NS5B polymerase inhibitor sofosbuvir came out, and then a lot of novel DAAs including NS5A protein inhibitors followed. Their emergence revolutionized the treatment of HCV infection. Now the IFN-free, DAA-based combination therapy has become standard therapeutic regimen for all the patients infected with HCV of various genotype (genotypes 1–6). Treatment

with this regimen for 12 weeks could achieve SVR rate of more than 95%, a long-term complete or partial clinical response and quite low rate of SAEs (less than 10%, even less than 1% in some regimens) in the patients with HCV-related Cryo Vas with or without GN.[72-80]

When Cryo GN is treated with DAA-based combination regimens, attention should be paid to the effects of renal dysfunction on DAAs clearance. Now it has been known that the NS3/4A inhibitors including paritaprevir, grazoprevir. Glecaprevir and asunaprevir, the NS5A inhibitors including daclatasvir, ombitasvir, elbasvir ledipasvir, and pibrentasvir, as well as the NS5B inhibitor dasabuvir all do not need dosage modification in renal insufficiency, even when GFR less than 10 mL/min. However, when GFR is less than 30 mL/min, the NS5B inhibitor sofosbuvir needs to reduce dosage or prolong the interval of administration. At the same GFR level, the dosage of the NS3/4A inhibitor simeprevir does not need to be adjusted, but should be used with caution.[57,73,81]

Before the end of this paragraph, we would like to emphasize that Cryo Vas is a disease which involves many disciplines, so its diagnosis and treatment require multidisciplinary collaboration. In particular, in the treatment of some highly specialized underlying diseases, for example, hematological malignancies, consultation with the experts of related disciplines is necessary.

In summary, this review discussed immunotyping of CGs, clinical and laboratory manifestations of Cryo Vas and GN, pathological features of Cryo GN, and recent advances in treatment. In this review, special attention has been paid to the issues in clinical practice.

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Conflicts of interest

None.

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