The Histopathologic Spectrum of Cryofibrinogenemia in Four Anatomic Sites

Skin, Lung, Muscle, and Kidney

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Abstract

Although the histologic characteristics of cryofibrinogenemia have been described in skin lesions, the literature is largely devoid of descriptions of this disorder in other organs. This series is the first to document the histopathologic manifestations of intrapulmonary, intramuscular, and renal cryofibrinogenemia. We describe the histopathologic manifestations of cryofibrinogenemia in 10 cases with manifestations in 4 organ systems: skin in 7 cases, skeletal muscle in 2, lung in 2, and kidney in 1.

Irrespective of anatomic site, all lesions showed an occlusive thrombotic diathesis comprising eosinophilic refractile deposits within vessel lumina with extension into the intima, with or without an accompanying characteristic granulomatous vasculitic component. Ultrastructural examination of the renal deposits showed fibrillary material within glomerular capillary lumina with unique morphologic features not previously described. Analysis of plasma from several cases revealed a cold-precipitable protein, which in most cases included a monoclonal paraprotein. The laboratory and histologic distinctions between cryofibrinogenemia and cryoglobulinemia are addressed. We provide guidelines for the proper handling of patient specimens in the workup of cryofibrinogenemia.

Cryofibrinogenemia refers to the presence in plasma of cryoproteins mainly comprising fibrinogen, fibrin, and fibrin split products, which may be primary, with no identifiable cause, or secondary to certain autoimmune, malignant, thrombotic, inflammatory, and infectious disorders.1-3 Although the pathogenesis of cryofibrinogenemia is not well understood, the associated cutaneous and systemic manifestations have been documented.1-3 The literature describing the histopathologic manifestations of cryofibrinogenemia is limited, with most reports being in the context of skin lesions; cryofibrinogenemia-associated lesions of other organ systems are not described. We describe the histopathologic manifestations in 10 patients with cryofibrinogenemia from 4 separate sites, namely, skin, muscle, lung, and kidney.

The aim of our study was to detail the histologic manifestations of cryofibrinogenemia in the skin and in organ sites not previously described. Also included are the novel ultrastructural findings of glomerular deposits seen in a renal biopsy specimen from 1 patient with cryofibrinogenemia. The distinction of cryofibrinogenemia from cryoglobulinemia is discussed as it pertains to histopathologic features and laboratory testing. The analysis of blood samples for cryoproteins requires that samples be kept at 37°C during the entire processing period to prevent false-negative results. We provide methodologic guidelines used in our laboratory for cryofibrinogen screening.

Materials and Methods

We prospectively encountered 10 patients with cryofibrinogenemia manifest in skin (7 cases), lung (2 cases), muscle (2 cases), and kidney (1 case) biopsy specimens. In all cases, we used light microscopy to study 5-µm, H&E- and periodic
acid–Schiff (PAS)-stained sections of paraffin-embedded, formalin-fixed tissue. Direct immunofluorescence (DIF) studies for IgG, IgA, IgM, and C3 were performed in 3 cases by overlay of a commercially prepared fluorescein-conjugated antibody on sections cut from frozen (−70°C) biopsy material and examined with a fluorescent microscope. An indirect immunofluorescence method with a fluorescein-conjugated, rabbit antimouse antibody was used to detect the presence of C5b-9 in frozen sections of these same 3 cases using anti-C5b-9 (aE11 clone, DAKO, Carpinteria, CA).

Serum and plasma samples were studied to confirm the presence and nature of the cryoprecipitate using the technique described in Appendix II.

Table 1
Clinical and Pathologic Data

<table>
<thead>
<tr>
<th>Case No./ Sex/Age</th>
<th>Medical History</th>
<th>Clinical Information/ Management</th>
<th>Biopsy Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/80 y</td>
<td>Peripheral neuropathy; refractory anemia; left-sided hemiparesis; chronic renal insufficiency; lvedo reticularis</td>
<td>Skin biopsy</td>
<td>Muscle: myocyte atrophy and necrosis; hypereosinophilic thrombi within endomyial vessels; focal granulomatous, necrotizing vasculitis of small arteries</td>
</tr>
<tr>
<td>2/M/78 y</td>
<td>Hypertension; seizures; degenerative joint disease; dementia; palpable purpura; ecchymosis; skin necrosis of toes</td>
<td>Skin biopsy</td>
<td>Skin: subcutaneous thrombogenic lymphocytic vasculopathy</td>
</tr>
<tr>
<td>3/F/41 y</td>
<td>Renal insufficiency; type 1 diabetes mellitus; hypothyroidism; neuropathy; deep venous thrombosis; visual disturbances; bilateral lower extremity swelling and weakness</td>
<td>Skin biopsy; plasmapheresis</td>
<td>Skin: pauci-inflammatory thrombogenic vasculopathy reaction pattern with vascular deposition of eosinophilic debris; focal neutrophilic vascular infiltrates</td>
</tr>
<tr>
<td>4/M/74 y</td>
<td>Coronary artery atherosclerosis; coronary artery bypass graft placement (remote); hypertension; chronic obstructive pulmonary disease; left eye blindness secondary to anterior ischemic ophtalmic; preler hypereosinophilic thrombus; acute respiratory and renal failure; pneumonia</td>
<td>Open lung biopsy; plasmapheresis</td>
<td>Kidney: hypereosinophilic nodular deposits in the mesangium, glomerular capillaries, and renal tubules</td>
</tr>
<tr>
<td>5/M/42 y</td>
<td>Cryofibrinogenemia; ischemic vasculitis; arthropathy; peripheral neuropathy; lower extremity rash</td>
<td>Skin biopsy; plasmapheresis</td>
<td>Skin: vascular occlusion by eosinophilic, PAS-positive precipitates with vascular infiltration by monocellular cells</td>
</tr>
<tr>
<td>6/F/32 y</td>
<td>Painful violaceous nodules on toes for 1 y</td>
<td>Skin biopsy; skin nodules temporally associated with antecedent streptococcal infection</td>
<td>Lung: diffuse septal capillary vasculopathy with luminal and intramural deposition of hypereosinophilic material within venules, arterioles, septal capillaries, and alveolar spaces; interstitial foreign body–type giant cell response; interstitial and intra-alveolar red cell extravasation and hemosiderin deposition; plasmacellular, interalveolar infiltrate with occasional atypical plasma cells</td>
</tr>
<tr>
<td>7/M/70 y</td>
<td>Coronary artery atherosclerosis; type 1 diabetes mellitus; hypertension; gout; intermittent hemophyisis for 18 mo; new onset renal insufficiency</td>
<td>Open lung biopsy; hemoptysis cleared with oral steroids</td>
<td>Skin: thrombogenic vascular reaction pattern with intense lymphocytic infiltrate, lymphocytic eccrine hidradenitis, and ischemic changes of epidermis</td>
</tr>
<tr>
<td>8/F/8 mo</td>
<td>Spinal muscular atrophy; ventilator dependent since birth; cutaneous ulcers and purpuric nodules on feet; spinal muscular atrophy; recurrent respiratory infections</td>
<td>Skin biopsy; numerous recent respiratory infections</td>
<td>Lung: intra-alveolar hemorrhage with deposition of IgM, IgA, C1Q, and C5b-9 by DIF and IIF studies</td>
</tr>
<tr>
<td>9/F/42 y</td>
<td>End-stage renal disease; type 1 diabetes mellitus; hypertension; coronary artery atherosclerosis; bilateral quadriceps and calf pain for 1-2 wk</td>
<td>Muscle biopsy, left thigh</td>
<td>Skin: occlusive eosinophilic deposits within blood vessel lumina and walls; variable inflammatory infiltrate of lymphocytes and histiocytes</td>
</tr>
<tr>
<td>10/F/61 y</td>
<td>Chronic renal failure; peripheral vascular disease; coronary artery atherosclerosis; chronic obstructive pulmonary disease; hypertension; lvedo reticularis; skin necrosis of bilateral lower extremities</td>
<td>Skin biopsy</td>
<td>Muscle: severe ischemic myopathy with myocyte atrophy and necrosis; pauci-inflammatory thrombotic vasculopathy with occlusion of endomyial capillaries by hypereosinophilic fibrin thrombi; focal granulomatous response of small arteries</td>
</tr>
</tbody>
</table>

In 6 cases, immunofixation electrophoresis was performed on the cryoprecipitate. After the cryoprecipitate was electrophoresed on an SPIFE IFE gel (Helena Laboratories, Beaumont, TX) to resolve the constituent proteins, antisera to human IgG, IgA, IgM, kappa light chains, and lambda light chains were applied.

### Results

#### Clinical Data

The 10 patients comprised 5 males and 5 females aged 8 months to 80 years Table I. The patients with dermatologic
manifestations of cryofibrinogenemia had a spectrum of clinical signs and symptoms, including livedo reticularis, purpuric nodules, and skin necrosis. Others with sequelae of cryofibrinogenemia in skin, other anatomic locations, or both had symptoms of pain and swelling of the extremities, arthralgias, and myalgias. Several patients had peripheral neuropathies, renal disease or failure, or pulmonary insufficiency or failure. None of the patients had an underlying hematologic malignant neoplasm.

Pathologic Findings

All skin biopsy specimens (Table 1) in cases 1, 2, 3, 5, 6, 8, and 10 showed a similar pattern of occlusive eosinophilic deposits within the blood vessel lumina and walls with a variable inflammatory infiltrate of lymphocytes and histiocytes. Compared with fibrin precipitates, the deposits were more eosinophilic and had a dense, almost refractile quality. In addition, the deposits assumed a retracted position in the vessel lumen and were encased in proplastic-appearing mononuclear cells of probable endothelial cell derivation. As well, there were admixed intraluminal histiocytes containing engulfed precipitates, focally imparting hypercellularity to the thrombus. The intravascular precipitates also stained more intensely with PAS compared with fibrin. In case 6, the histologic features resembled pernio by virtue of an intense lymphocytic vascular reaction with lymphocytic eccrine hidradenitis. Ischemic changes were seen in the epidermis supplied by the occluded vessels in case 2. In case 2, focal angiocentric infiltrates of neutrophils with striking leukocytoclasia, along with segmental mural fibrin deposition, produced morphologic features closely recapitulating leukocytoclastic vasculitis, although in other areas the findings were those of a nonnecrotizing pauci-inflammatory thrombogenic vasculopathy. In cases 1 and 5, the histiocytic nature of the mural infiltrate imparted granulomatous morphologic features to the vasculopathy. In addition, in case 1, there was a prominent angiocentric lymphocytic infiltrate. DIF in cases 1 and 5 showed intense IgG and C5b-9 deposition within the intraluminal deposits, along with focal weak staining of IgM.

In case 4, representing cryofibrinogenemia involving the lung, there was an extensive organizing pneumonitis and bronchiolitis resulting in a deceptive low-power architecture compatible with bronchiolitis obliterans with organizing pneumonitis. However this process was associated temporally with microvascular injury, hallmarks of which included interstitial and intra-alveolar red cell extravasation and hemosiderin deposition. In addition, there were hypereosinophilic precipitates within the venules, arterioles, septal capillaries, and alveolar spaces. The precipitates also were present within the interstitium, with varying degrees of nuclear atypia. Rare plasma cells contained rhomboidal and rectangular crystals. Oil-immersion examination (×1,000 magnification) showed disrupted septal capillary basement membranes accompanied by occlusive luminal and intramural precipitates, the latter being hypereosinophilic and assuming rhomboidal and/or cylindrical shapes. A DIF preparation demonstrated IgG and IgM within the precipitates. The second pulmonary case (case 7)
demonstrated intra-alveolar hemorrhage; DIF studies showed extensive deposits of IgM, IgA, and complement.

The muscle biopsy specimens in cases 1 and 9 exhibited ischemic alterations characterized by myocyte atrophy and necrosis. The endomysial vessels manifested an occlusive thrombotic diathesis consisting of hypereosinophilic thrombi with an apparent extension of the precipitates into the intima resulting in a concentric hypereosinophilic expansile alteration of the intima.[Image 7] and [Image 8]. In both cases, occasional small arteries showed a granulomatous response in association with hypereosinophilic mural deposits.

In case 3, the kidney showed hypereosinophilic nodular deposits in the mesangium and also within the glomerular capillaries.[Image 9]. Similar deposits were present in the renal tubules.[Image 10]. Ultrastructural examination of a glomerulus showed typical changes of advanced diabetic glomerulopathy. There was prominent thickening of glomerular basement membranes, more than 1,400 nm in many areas. There also was a marked nodular accumulation...
of basement membrane–like material within the mesangium. In addition to these changes, however, many of the glomerular capillary lumina contained aggregates of fibrillary material on ultrastructural examination **Image 11** and **Image 12**. These fibers, which measured approximately 20 nm in diameter, were arranged in a haphazard orientation in some aggregates and a more parallel array in others. These fibrillary aggregates were distinct from the glomerular basement membrane and the basement membrane–like mesangial stroma. Tactoids of fibrin were not seen.

Immunofixation electrophoresis results on the cryoproteins of cases 1, 3, 4, 5, and 7 each revealed a monoclonal, IgG kappa paraprotein. Immunofixation on case 8 revealed a polyclonal pattern.

**Discussion**

Cryofibrinogenemia refers to the presence in the plasma of a cold-precipitable protein composed of fibrinogen, fibrin, fibrin fragments X and Y, fibronectin, albumin, factor VIII,
small amounts of immunoglobulins, and other plasma proteins.\textsuperscript{2,4-6} The pathogenesis of cryofibrinogenemia is not well understood. Cryofibrinogenemia is divided broadly into 2 categories, primary and secondary, the former occurring without evidence of an inciting disease process. Secondary disease has been linked to such conditions as multiple myeloma, carcinoma (eg, pulmonary, ovary, gastric, and prostate), leukemia, fibrosarcoma, lymphoma, autoimmune disease, chronic hepatitis, collagen vascular disease, and infectious stimuli.\textsuperscript{2,7-9} Immunofixation conducted on 5 of our cases revealed an IgG kappa monoclonal protein. This finding was suggestive of a low-grade lymphoproliferative process of B-cell lineage, which can be associated with cryofibrinogenemia, although none of the patients in our study had a hematologic malignant neoplasm. In 1 other case, the cryofibrinogen was associated temporally with a recent streptococcal infection as evidenced by high anti-streptolysin O titers.

Clinical manifestations of cryofibrinogenemia are most prominent in the skin and include cold sensitivity, acral purpura, hemorrhagic necrosis, and gangrene. The prevalence of cryofibrinogens in the general population has been noted to be 2.8%.\textsuperscript{2} Clinical symptoms or signs commonly attributed to cryoproteinemia have been noted in approximately 10% of patients with cryofibrinogenemia.\textsuperscript{9} Frequent presenting symptoms include cold sensitivity, purpura, livedo reticularis, Raynaud phenomenon, skin ulceration, gangrene, and skin necrosis.\textsuperscript{1} Typically the lesions arise on the distal extremities, ears, and nose. The cutaneous findings are a result of ischemic damage due to deposition of the precipitating proteins within the dermal vasculature, induced by a drop in temperature below 37°C. Many of our patients had some of these classically described dermatologic manifestations of cryofibrinogenemia, including livedo reticularis, skin necrosis, and purpuric nodules. Other clinical signs and symptoms noted in several of our patients likely reflect the sequelae of ischemic changes (acute and/or chronic) in end organs, for example, acute respiratory failure in case 4 and renal failure in cases 1, 3, 7, 9, and 10. Other signs and symptoms that have been associated with cryofibrinogenemia such as neuropathy, arthralgias, and myalgias were seen in many of our patients.\textsuperscript{8}

Classically, the histomorphologic features of cryofibrinogenemia as it affects the skin have been described as occlusion of the superficial and/or deep vascular plexuses by eosinophilic, globular material with a variable lymphocytic infiltrate surrounding and permeating the vessel wall.\textsuperscript{1,4,5,7,9,10} We found that regardless of anatomic location, the histologic manifestations of cryofibrinogenemia appeared somewhat similar and reflected the common underlying process of vascular occlusion secondary to precipitation of the cryoprotein within vascular spaces. Rarely is a true vasculitis present, reflecting an Arthus type III immune complex reaction due to antifibrinogen antibodies complexed to fibrinogen. The precipitates are hyper-eosinophilic and can assume a geometric configuration within the vascular lumen; one study found that the precipitates typically assumed a cylindrical configuration,\textsuperscript{9} a finding we encountered in the lung biopsy specimen of case
In our cases, evidence suggestive of the diagnosis of cryofibrinogenemia included the hypercellular nature of the thrombi due to admixed macrophages containing engulfed precipitates, the encasement of the intraluminal precipitates by hyperplastic endothelial cells, and the granulomatous response associated with the intraluminal and intimal precipitates.

The absolute histopathologic distinction between cryoglobulinemia and cryofibrinogenemia may be difficult, as both can be associated with hypereosinophilic precipitates highlighted by the PAS stain. However, there are important clues that may suggest the presence of cryofibrinogens. First, in cryofibrinogenemia, the precipitates have a tendency to evoke a granulomatous response, presumably reflecting a foreign body reaction to the material. Hence, both extravascular granulomatous foci and granulomatous vasculopathy are characteristic. Typically, cryoglobulinemia elicits leukocytic, lymphocytic, or mixed vasculitis. Hyaline thrombosis was noted by Cohen et al in 19% of cutaneous biopsy specimens from patients with cryoglobulinemia, and of these cases, only approximately 50% exhibited hyaline thrombosis as a predominant histologic feature. Also the cryofibrinogen precipitates often assume a characteristic cylindrical configuration within the vascular lumina, as noted in several of our cases. Another histopathologic characteristic ascribed to cryoglobulinemia is inflammatory or noninflammatory purpura without vasculitis, which was not encountered in any of our cases.

The histopathologic features attributable to cryofibrinogenemia in organ systems other than the skin are not well documented. We encountered 5 patients with diagnoses of cryofibrinogenemia who first manifested evidence of the disease at extracutaneous organ sites. A 74-year-old man (case 4) with respiratory failure, whose lung biopsy specimen is described in the preceding text, and a 70-year-old man (case 7), who had intermittent hemoptysis, represent the first documentation of the pulmonary pathology of cryofibrinogenemia. In the lung, there was extensive red cell extravasation with basement membrane zone disruption, accompanied by occlusive, hypereosinophilic deposits within venules, arterioles, septal capillaries, and alveolar spaces. In both cases, there was an associated organizing pneumonitis, which could obscure the diagnosis if the temporal association of the intra-alveolar fibromucinous polyps with microvascular injury is not noted.

Two other patients had muscle weakness secondary to an ischemic myopathy due to vascular occlusion with cryoproteins accompanied by a concomitant granulomatous vasculopathy, representing the first histologic documentation of cryofibrinogenemia in muscle.

To our knowledge, this series also represents the first description of renal involvement in a patient with a multisystem disease attributed to thrombo-occlusive aggregates of cryofibrinogen. The light microscopic appearance of the kidney most closely resembled light chain nephropathy manifesting as striking mesangial and capillary hypereosinophilic nodular deposits distorting the glomerular architecture. Nagy et al described the presence of detectable levels of cryofibrinogen in 74% of a cohort of patients with IgA nephropathy. They showed a correlation between the presence of circulating cryofibrinogen and the existence of IgG in glomeruli, and fibrin and fibrinogen in glomeruli and interstitium as studied by immunofluorescence. No distinctive intraluminal fibrillary material was reported in these patients.

Ultrastructural examination of a glomerulus from one of our cases showed typical changes of advanced diabetic glomerulopathy with marked thickening of glomerular basement membranes in excess of 1,400 nm. There also was marked nodular accumulation of basement membrane–like material within the mesangium. In addition to these changes, however, many of the glomerular capillary lumina contained aggregates of fibrillary material. The fibers, which measured approximately 20 nm in diameter, were arranged in both a haphazard orientation and a parallel array. These fibrillary aggregates were distinct from the glomerular basement membrane and the basement membrane–like mesangial stroma. They are morphologically unique and, to our knowledge, have not been described in the renal literature. By ultrastructural examination, this material is distinct from the 2.5-nm curved cylinders seen in the electron-dense deposits of cryoglobulinemia. While cryoglobulin deposits can be found within the glomerular capillary lumina, these most often are seen in a subendothelial location. This material also can be distinguished from the material that accumulates in fibrillar glomerulopathies, such as fibrillary glomerular nephritis and immunotactoid glomerulopathy. In these lesions, a fibrillary material accumulates that thickens the basement membrane of the glomeruli. In addition, the tubules in immunotactoid glomerulopathy are considerably larger (ie, >30 nm) than those seen in this case. Amyloid fibrils also can be distinguished from this material, not only by their smaller diameter (8-10 nm) but also by their location, which typically involves the mesangium and the glomerular basement membrane rather than the capillary lumina.

The DIF and indirect immunofluorescence studies performed in several of our cases showed IgG, IgM, C3, and C5b-9 vascular deposition compatible with humorally mediated microangiopathy. Five patients had features of vasculitis confirmed on biopsy and/or suggested by the presence of a pulmonary hemorrhage-renal syndrome. All 5 patients had a monoclonal paraprotein of the IgM subtype, as demonstrated by immunofixation electrophoresis. Because vasculitis is a characteristic hallmark
of type II cryofibrinogenemia, we hypothesize that these patients had type II cryofibrinogenemia comprising fibrinogen complexed to an IgM monoclonal paraprotein with antifibrinogen activity. A virtually identical phenomenon is seen with type II cryoglobulinemia, whereby a monoclonal antibody with rheumatoid factor activity binds to the Fc portion of IgG and pathologically deposits within vascular spaces. All of these patients have, in essence, a form of B-cell lymphoproliferative disease. Euler et al. described a patient with a monoclonal antifibrinogen antibody that precipitated on cooling only when fibrinogen was present. The antibody described was noted to precipitate when incubated with the patient’s fibrinogen, fibrinogen from a healthy unrelated donor, and fibrinogen from a pooled donor preparation, thereby demonstrating the fibrinogen specificity of the antibody-mediated cryoprecipitation. Also, the precipitate described by Euler et al. was unlike a typical cryofibrinogen precipitate composed of fibrin, fibrinogen, fibronectin, and coprecipitated immunoglobulins, in that it was composed of an immune complex of fibrinogen and the antifibrinogen antibody. A subsequent thorough hematologic evaluation disclosed no evidence of frank lymphoma or leukemia in any case in our series. In some cases of cryofibrinogenemia, however, there may be an underlying B-cell lymphoma.

The laboratory distinction of cryofibrinogens from other cryoproteins, primarily cryoglobulins, is critical. Cryoglobulins are serum-based (as opposed to plasma-based) cryoproteins that precipitate on cooling to 4°C. Cryoglobulins are associated with a similar spectrum of conditions; hence, the main associations are with lymphoproliferative, autoimmune, and infectious disorders. Although cryoglobulins may be demonstrated in cooled plasma, as well as serum, cryofibrinogens are present only in plasma; short of electrophoretic means, this characteristic permits distinction between the two. Both cryofibrinogens and cryoglobulins resolubilize on rewarming. To investigate the nature of the cryoprotein, plasma and serum samples must be analyzed. In all of our cases, the specimens were incubated at 4°C for 48 hours. No demonstrable precipitate was seen in the serum sample, while the plasma-containing tube was cloudy with a distinct precipitate at the bottom.

The diagnosis of cryofibrinogenemia rests on the identification of the plasma-based proteins, which precipitate at 4°C. To identify these proteins, proper handling of the specimen must be ensured (Appendix 1). The specimen must be obtained in a citrate, oxalate, or EDTA tube, not in a heparin tube. Many components of cryofibrinogens are consumed in the clotting process and, therefore, are not present in serum. Heparin has been shown to form a cryoprecipitate with fibronectin, fibrin, and fibrinogen (coined the “heparin-precipitable fraction”) and cause a false-positive result.

After collection, the specimen must be kept at 37°C to prevent precipitation of any plasma proteins. False-negative results may occur if the cryofibrinogen is allowed to cool, as it may autoabsorb to cooled RBCs. It is recommended that the specimen be centrifuged promptly after collection and that centrifugation be performed at 37°C. The plasma then is drawn off the specimen and incubated at 4°C for 48 to 72 hours. We have found that using a heated centrifuge is critical to the establishment of a cryoprecipitate. All specimens centrifuged in a room temperature centrifuge gave false-negative results despite the presence of a substantial cryoprecipitate, the presence of which was noted after using a heated centrifuge.

Although most patients with cryofibrinogenemia are asymptomatic, thrombomembolic events manifest in some; signs and symptoms include those already listed and constitutional symptoms such as fever, myalgias, and arthralgias. The mechanism for the onset of a thrombophilic state is poorly understood, but it has been noted that in patients with cryofibrinogenemia, serum levels of alpha1-antitrypsin and alpha2-macroglobulin are increased. Each of these serum elements functions as a serum protease with inhibitory activity on plasmin, a fibrinolytic serum protein. By inhibiting fibrinolysis, alpha1-antitrypsin and alpha2-macroglobulin cause the accumulation of the cryofibrinogen complex, which then leads to precipitation and deposition in vascular lumina, with subsequent manifestations of disease.

Treatment of cryofibrinogenemia is mostly symptomatic in cases of primary cryofibrinogenemia, and in secondary cases, it is aimed at the underlying cause. In addition, because of the association with underlying hematologic dyscrasia in the majority of cases, a thorough exploration of the patient’s hematologic status must be conducted, including in the context of a bone marrow biopsy. Patients historically have been treated with plasmapheresis and fibrinolytic agents such as streptokinase, streptodornase, and urokinase. These modalities are beneficial only in the short term owing to their cost and impracticality of long-term use. Several articles have described the efficacy of stanozolol, an androgenic steroid with fibrinolytic activity, in patients with cryofibrinogenemia. The mechanism of action is not well understood, but it is hypothesized to alter liver production of fibrinolytic proteins.

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Appendix II
Cryofibrinogen Screen

Principle: The patient’s plasma is screened for the presence of protein precipitating in the cold. Cryofibrinogen is precipitated from plasma when cooled at 4°C for 48 hours. This physical characteristic usually is reversible and disappears on warming.

Procedure:
1. Patient and normal control plasma are collected in 3 tubes and kept at 37°C. Samples are obtained in the following tubes:
   a. Red-top (allow to fully clot, approximately 30 min)
   b. Blue-top (3.8% citrate)
   c. Green-top
2. Spin all tubes at 37°C, 10 min, 3,000 rpm.
3. Using a Pasteur pipette, fill Winthrop tube with plasma or serum and clearly label tubes to differentiate between the tubes.
4. Incubate the excess plasma or serum and Winthrop tubes at 4°C for 48 h.
5. If the specimen is positive for cryoprecipitated globulin, a fine white precipitate will be seen in the bottom of the cooled tubes. When a cryofibrinogen is present, there will be significantly more precipitate present in the plasma from the green-top tube, followed by a decreasing amount in the plasma from the blue-top tube, and none present in the serum from the red-top tube.
6. Spin tubes at 4°C, 10 min, 3,000 rpm.

Normal result: Negative

References