Cryoglobulins (CGs) are serum proteins that reversibly precipitate when the serum is cooled below 37°C. Cryoglobulinemias are associated with a variety of diseases, including hematologic, autoimmune, and infectious. Isolation of CGs requires handling the specimen with extreme care. We describe a 70-year-old man, recently diagnosed with chronic lymphocytic leukemia, who developed hemorrhagic crusted skin ulcers on his legs that were pruritic and painful. Results of skin biopsies showed dilated superficial, mid-dermal and deep-dermal blood vessels containing pink amorphous material and red blood cells. Cryoglobulinemia was suspected; however, an initial search for CGs was negative. There was concern about suboptimal handling of the specimen, and the test was repeated. Two percent CGs with IgM κ specificity were detected. This case illustrates the importance of the proper handling of specimens for evaluation of cryoglobulinemia.
Cryoglobulins (CGs) are serum immunoglobulins that reversibly precipitate when serum is cooled below 37°C. Cryoglobulinemia has been classified into 3 types. Cryoglobulinemia may affect a variety of organs, including skin, which may help in the early recognition of this disease. The isolation of CGs requires careful handling of the specimen. We report a case of type I cryoglobulinemia presenting as hemorrhagic crusted cutaneous ulcers on the legs of a patient with early chronic lymphocytic leukemia.

**Case Report**

A 70-year-old man presented with a 6-month history of pruritic and painful erosions and shallow ulcerations that began on his lower legs. The lesions progressed over time to extend to his thighs, lower trunk, and upper extremities. The patient also noted discoloration of his nails, but his upper trunk, head, and neck were spared. He complained of pruritus, discomfort, pain, and difficulty sleeping. He had no fever, chills, weight loss, or shortness of breath. The patient had been recently diagnosed with chronic lymphocytic leukemia; however, he was not receiving any therapy at the time of presentation.

Results of a physical examination revealed multiple diffuse shallow ulcers rimmed by erythema and covered with black crusted eschars (Figure 1). These lesions were symmetrically distributed on his lower legs. All were moderately tender. His peripheral pulses were intact. There was mild diffuse erythema and slight induration of the skin on the thighs. The upper extremities were minimally involved. All his fingernails had a violaceous subungual discoloration, which became dark brown to black as time passed (Figure 2).

Results of laboratory evaluations revealed a red blood cell (RBC) count of 3.94×10⁶/µL (reference range, 4.20–5.80×10⁶/µL); hemoglobin level, 11.3 g/dL (reference range, 13.1–17.1 g/dL); hematocrit level, 33% (reference range, 40%–51%); platelet count, 465×10³/µL (reference range, 140–400×10³/µL); and white blood cell count, 10,600/µL (reference range, 3900–11,400/µL), although it had been 2670/µL with 7% bands 11 days prior to that. His electrolytes were within reference range. Lactate dehydrogenase was 281 U/L (reference range, 60–245 U/L). Recent prothrombin time and partial thromboplastin time also were within reference range. Erythrocyte sedimentation rate was 124 mm/h (reference range, 0–20 mm/h). Results of a hepatitis C virus (HCV) antibody test were negative. Immunofixation and protein electrophoresis showed a normal profile. Results of initial CG studies were negative; however, repeat testing while the patient was receiving oral prednisone detected 2% CGs with IgM κ specificity.

Several biopsies were obtained from the lower extremities, all of which had a similar microscopic appearance (Figure 3). The epidermis was focally necrotic. There was dilatation of the superficial, mid-dermal, and deep-dermal vascular plexus. The vessels contained pink amorphous material and RBCs. There was some extravasation of RBCs into the dermis and no evidence of vasculitis. CD31 immunohistochemical stain outlined the vascular channels, confirming that the eosinophilic material was, indeed, in vascular spaces. The eosinophilic material stained for both κ and λ light chains, with more prominent κ staining. Phosphotungstic acid hematoxylin stained sections did not reveal fibrin deposits within the vascular structures.
CGs were first described by Wintrobe and Buell in 1933 in a patient with multiple myeloma. Brouet et al. studied 86 patients with cryoglobulinemia and classified CGs into 3 types. Type I CGs are monoclonal CGs composed of IgM, IgG, IgA, and Bence Jones proteins in decreasing order of frequency of occurrence. The serum level of monoclonal CGs is typically high, and they precipitate easily at cold temperatures. Type II CGs are mixed CGs composed of complexes of polyclonal IgG and monoclonal IgM rheumatoid factor. The serum concentration of type II CGs is typically high, but the monoclonal component may be too low to be detected with serum protein immunoelectrophoresis. Type III CGs are mixed polyclonal CGs, most commonly composed of IgM and IgG. They are usually present at very low levels in the serum. Brouet et al. found type I CGs in 24.5%, type II in 25.5%, and type III in 50% of cases of cryoglobulinemia.

Type I cryoglobulinemia is associated with multiple myeloma, other hematologic proliferative disorders, rheumatoid arthritis, and autoimmune hemolytic anemia. Type II and III cryoglobulinemias are associated with chronic lymphocytic leukemia or lymphoma. Autoimmune diseases, such as Sjögren syndrome and rheumatoid arthritis, are found in patients with type II cryoglobulinemia. Type III cryoglobulinemia is seen in patients with systemic lupus erythematosus, periarteritis nodosa, idiopathic thrombocytopenic purpura, and hematologic proliferative disorders. Infections, including hepatitis B, mononucleosis, Cytomegalovirus, subacute bacterial endocarditis, and toxoplasmosis, can be found in patients with cryoglobulinemias. The term essential cryoglobulinemia describes cryoglobulinemia with no clinical signs of underlying disease; however, HCV infection has been found in many patients with mixed essential cryoglobulinemia. Agnello et al. found HCV antibodies in 8 of 19 and HCV RNA in 16 of 19 patients with type II cryoglobulinemia.

Clinical presentation of patients with cryoglobulinemia includes purpura, arthralgia, and fatigue. A minority of patients present with vasculitis affecting the kidneys, skin, liver, brain, or abdominal organs. Skin involvement may help in the early recognition of the disease. Brouet at al. found cutaneous lesions or vasomotor symptoms in two thirds of patients. Leg ulcers were seen in 8% of patients with type I cryoglobulinemia, 0% of patients with type II, and in 4% of patients with type III. Renal and neurologic manifestations, hemorrhages, abdominal pain, and arterial thromboses are among the other findings.

Cohen et al. studied 72 patients with cryoglobulinemia and found that skin ulcers and hemorrhagic crusts or bullae were more common in patients with type I cryoglobulinemia. In patients with cryoglobulinemia who had cutaneous lesions, lower extremities were the most commonly affected site, being involved in 100% of patients with types II and III and in 92% of patients with type I. Other affected sites were upper extremities (39%), trunk (29%), head and neck (15%), oral mucosa (7%), and nasal mucosa (7%). Bork et al. reported an uncommon case of a 62-year-old man with IgG-λ multiple myeloma and cryoglobulinemia.
who developed follicular hyperkeratotic spicules secondary to IgG dysprotein and cryoglobulin accumulating in follicular epithelium, which disturbed normal keratinization.

Histopathologic findings in patients with cryoglobulinemia include vasculitis, inflammatory and noninflammatory purpura, noninflammatory hyaline thrombosis, postinflammatory sequelae, and necrobiotic xanthogranuloma. Vasculitis, primarily leukocytoclastic, is seen almost equally in all types of cryoglobulinemia and is most commonly associated with low levels of CGs. Vasculitis manifests initially as purpura, petechiae, ulcers, livedo reticularis, scarring, and hemorrhagic crusts. Of the 72 biopsy specimens studied by Cohen et al, 10% (7) showed noninflammatory hyaline thrombosis, a finding that was more frequent and pronounced in type I cryoglobulinemia and more commonly associated with high CG levels. Ultrastructural studies of a skin biopsy in monoclonal IgG, cryoglobulinemia-associated dermatitis showed large amounts of fibrin within capillary lumina and intraluminal crystalloid structures of different shapes and sizes. Results of immunofluorescence revealed positive intravascular staining for IgG, C3, and fibrin.

Suggested investigations of patients suspected of having cryoglobulinemia include laboratory evaluations of cryocrit levels, erythrocyte sedimentation

Figure 3. Low-power view (A) shows focal epidermal necrosis and dilatation of the superficial, mid-dermal, and deep-dermal blood vessels. The blood vessels contain pink amorphous material (H&E, original magnification ×4). High-power view (B) shows dilated dermal blood vessels containing pink amorphous material and red blood cells (H&E, original magnification ×40).
rate, rheumatoid factor, and early complement components C2 and C4. Biopsy of the affected organ, such as skin, and a search for underlying disease, such as HCV, are recommended. Extreme care should be taken when isolating CGs. Temperatures at which CGs start to precipitate may be as high as 35°C. For this reason, blood should be drawn using syringes prewarmed to 37°C and maintained at body temperature during transport. Blood should be collected in tubes without anticoagulant and allowed to clot at 37°C. The serum is incubated at 0°C to 4°C for 5 to 7 days, then centrifuged. The packed volume of precipitate is the cryocrit. Generally, monoclonal CGs precipitate at relatively high temperatures within 24 hours, while mixed CGs, particularly type III, are present at low concentrations and precipitate at low temperatures after a prolonged period of time. Serum protein electrophoresis and immunoelectrophoresis are utilized for classification of CGs. The course and prognosis of cryoglobulinemia is related to the presence of underlying disease, such as a lymphoproliferative disorder. The presence of renal disease has an adverse effect on survival.

In this case, an initial search for CGs was negative; however, because cryoglobulinemia was clinically suspected, there was concern that the specimen was incorrectly handled. Repeat CG studies were obtained while the patient was already being treated with oral prednisone, revealing the presence of CGs. This case illustrates the importance of the proper handling of specimens for evaluation of cryoglobulinemia.

REFERENCES
1. Wintrobe MM, Buell MV. Hyperproteinemia associated with multiple myeloma, with report of a case in which an extraordinary hyperproteinemia was associated with thrombosis of the retinal veins and symptoms suggesting Raynaud’s disease. Bull Johns Hopkins Hosp. 1933;52:156-165.