Cryoglobulins are immunoglobulins that precipitate as serum is cooled below core body temperature. Adequate characterization requires repeated washing of cryoprecipitates to remove nonspecifically adherent serum proteins; following such processing the cryoglobulin can be resolubilized by warming again to 37°C. Simple, or single-component, cryoglobulins contain only 1 isotype or subclass of immunoglobulin or, in rare instances, a cryoprecipitable monoclonal immunoglobulin light chain. Mixed cryoglobulins are immune complexes composed of 2 different types of immunoglobulin, most commonly an IgM-antiglobulin (the antibody) and IgG (the antigen). The classification initially proposed by Brouet et al.

The frequency with which the different types of cryoglobulins are seen in a reference laboratory varies according to the type of patient population sampled and how carefully cryoprecipitates are characterized. Tertiary care facilities servicing patients with known gammapathies, plasma cell dyscrasias, or lymphoproliferative diseases are more likely to see type I or type II cryoglobulins, whereas referral centers for rheumatological disorders or nephritis will see more patients with type II or III. The reported incidence of type II cryoglobulins, many of which are associated with chronic hepatitis C virus (HCV) infection, has increased markedly during the past 10 years. Recent series have found up to approximately 80% of cryoglobulins to be type II, compared with 14% to 26% about 30 years ago. This change reflects (1) a heightened appreciation of the extrahepatic and autoimmune manifestations of HCV infection, as well as the possible association with low-grade lymphoma, and (2) the increased use of immunofixation and other sensitive methods for the detection of the low-level IgM component often found in these proteins.

Testing for cryoglobulinemia may be carried out in 2 stages. The first stage involves an initial screening for cryoprecipitation in serum, in which a precipitate is demonstrated to be primarily immunoglobulin and is largely resolubilized by warming. This step is most useful when coupled with quantitation of the level of cryoglobulin in the sample. The second stage requires more detailed characterization and typing of the cryoglobulin by immunofixation analysis and, in the case of type I and type II cryoglobulins, a review or performance of other standard laboratory testing for monoclonal gammapathy.

**INDICATIONS FOR TESTING**

Some centralized laboratories that service a large area may receive 400 to 500 serum samples per week for testing; these laboratories may encounter positive results for cryoglobulinemia in only 2% to 5% of these samples. By contrast, laboratories servicing rheumatology or hematology/oncology practices, or laboratories located in major tertiary referral centers, record a much higher frequency of positive tests. These differences are due to (1) varying indications by different clinicians for ordering a cryoglobulin screen and (2) the procedures used for collecting and processing serum.

Cryoglobulin testing should only be carried out if a patient has suggestive clinical symptoms or laboratory findings (Table 3) and is known to have a disease that may be
associated with cryoglobulinemia (Table 2). Such studies should be distinguished from tests of other cold-related phenomena or cryopathies, such as cold agglutinins or cryofibrinogenemia.1,2 Because mixed polyclonal cryoglobulins are often present at very low levels that approximate the limits of detection, random testing for type III cryoglobulins in particular is strongly discouraged.

**COLLECTION OF SERUM FOR TESTING**

Once the decision is made to test for cryoglobulins, it is critical to collect the serum properly. Common errors include loss of cryoglobulins due to failure to properly separate serum from whole blood, loss of cryoprecipitate due to refrigeration before centrifugation, and an inadequate volume of serum for testing of cryoglobulins present at low levels.

**Table 1. Types of Cryoglobulin***

<table>
<thead>
<tr>
<th>Simple (single-component) cryoglobulins (type I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
</tr>
<tr>
<td>IgM</td>
</tr>
<tr>
<td>IgA</td>
</tr>
<tr>
<td>Immunoglobulin light chain</td>
</tr>
<tr>
<td>Complexed to other proteins</td>
</tr>
<tr>
<td>Mixed cryoglobulins</td>
</tr>
<tr>
<td>IgM rheumatoid factors</td>
</tr>
<tr>
<td>Monoclonal (type II)</td>
</tr>
<tr>
<td>Polyclonal (type III)</td>
</tr>
</tbody>
</table>

* The M-components seen in types I and II cryoglobulinemias are most frequently IgM molecules, and it has been estimated that approximately 10% of all Waldenström's macroglobulins are cryoprecipitable. Immunoglobulin M type I cryoglobulins are approximately 1.5 times more prevalent than IgG type I cryoglobulins.3 Thus, significant skewing exists among type I cryoglobulins to the IgM isotype, especially considering that, overall, IgM accounts for only about 15% of gammopathies, a number that reflects closely the contribution of this iso-type to the total vascular body pool of immunoglobulins. This skewing toward IgM is even more striking among type II cryoglobulins, which are almost invariably IgM molecules utilizing the VKIII subgroup and selective V-region heavy- and light-chain genes.43

**Table 2. Disease Associations**

<table>
<thead>
<tr>
<th>Type I</th>
</tr>
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<tbody>
<tr>
<td>Multiple myeloma</td>
</tr>
<tr>
<td>Waldenström's macroglobulinemia</td>
</tr>
<tr>
<td>Other lymphoproliferative diseases with M</td>
</tr>
<tr>
<td>components</td>
</tr>
<tr>
<td>Type II</td>
</tr>
<tr>
<td>Chronic hepatitis C virus infection</td>
</tr>
<tr>
<td>Sjögren's syndrome</td>
</tr>
<tr>
<td>Waldenström's macroglobulinemia</td>
</tr>
<tr>
<td>Chronic lymphocytic leukemia</td>
</tr>
<tr>
<td>Non-Hodgkin's lymphoma</td>
</tr>
<tr>
<td>Autoimmune diseases</td>
</tr>
<tr>
<td>Cold agglutinin disease</td>
</tr>
<tr>
<td>Type III*</td>
</tr>
<tr>
<td>Chronic infections</td>
</tr>
<tr>
<td>Viral (EBV, CMV, HIV, hepatitis viruses)</td>
</tr>
<tr>
<td>Bacterial (SBE, leprosy, spirochetal)</td>
</tr>
<tr>
<td>Fungal, parasitic</td>
</tr>
<tr>
<td>Autoimmune diseases</td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
</tr>
<tr>
<td>Inflammatory bowel diseases</td>
</tr>
<tr>
<td>Biliary cirrhosis</td>
</tr>
</tbody>
</table>

* Many patients with type I cryoglobulins do not manifest specific symptomatology, suggesting that other factors may be important to the clinical expression of disease.

We recommend that 10 to 20 mL of blood be collected into a tube or syringe prewarmed to 37°C, and that the sample be allowed to clot at this temperature for 30 to 60 minutes prior to separation. Since cryoglobulin levels may vary from 50–100 μg/mL to 5–10 mg/mL or higher, the volume of serum necessary for complete characterization may vary by a factor of 100 to 200. If a high thermal amplitude cryoglobulin is suspected or has been detected previously, or if a cryogel is found, drawing blood into a prewarmed syringe or red top tube is especially important; alternatively, the sample can be drawn into a tube containing EDTA. In some centers, blood is placed in a thermostats filled with sand kept at 37°C prior to transfer to the laboratory for centrifugation; alternatively, samples may be placed in a water bath kept at this temperature in an outreach laboratory equipped with a centrifuge. Serum is separated from the clot by centrifuging warm for 10 minutes at 2500 rpm. Following separation, the serum is checked for lipemia, which may complicate visual inspection of the sample for cryoprecipitation. If prolonged delay is anticipated during shipment of samples, or if sterility is an issue, one drop of 0.1 g sodium azide per liter may be added to the sample to prevent bacterial overgrowth. Collection of blood from a patient receiving anticoagu-
lation therapy, particularly a patient on intravenous heparin or who has been significantly exposed to this agent during therapeutic plasmapheresis, presents specific problems in interpretation. The presence of fibrinogen may introduce an artifact in the β region of the protein electrophoresis that can be mistaken for a monoclonal protein, particularly if capillary electrophoresis is carried out. In this situation, thrombin may be added to the warm sample to induce clotting. Cryoprecipitation may also occur to complex formation between heparin and fibronectin; in addition, fibronectin itself is cold insoluble. Finally, some cryoproteins have been found to contain fibronectin, most likely through specific binding sites between this molecule and immunoglobulin.\textsuperscript{7,8} Cryoprecipitation may be due to cold-precipitable complexes of fibrin and fibrinogen (so-called cryofibrinogenemia) or heparin-precipitable fibrinectin complexes. Thus, in a patient receiving anticoagulant therapy, a cryoglobulin screen may be falsely positive, and immunochemical characterization of any cryoprecipitate obtained is necessary to confirm the presence of immunoglobulin.\textsuperscript{1,2}

**OBSERVATION FOR CRYOPRECIPITATE FORMATION**

Serum processed as described is tested for cryoprecipitation by daily inspection at 4°C for 7 days. Blood or serum should not be freeze-thawed at any point during processing, as this may significantly affect immunoglobulin solubility. In some laboratories, a parallel aliquot is kept at 37°C for comparison, and in others a third aliquot is kept at 4°C to be used pending a positive result for rigorous washing, resolubilization by warming, and repeat cryoprecipitation prior to detailed characterization. Each of these modifications have merit for initial processing of samples.

Cryoprecipitation is quite variable from one sample to another, depending on the type, concentration, and thermal amplitude of the cryoglobulin. Type I cryoglobulins, which are often present at concentrations exceeding 5 mg/mL, and type II cryoglobulins, which are frequently found in excess of 1 mg/mL, may begin to precipitate within hours of refrigeration and are usually apparent by the next day. By contrast, type III cryoglobulins, most of which are found at levels less than 1 mg/mL, may require several days to precipitate and may be barely visible at the base of the centrifuge tube. Most cryoglobulins are white and gelatinous, although type I cryoglobulins may occasionally appear flocculent or form crystals. The latter can be characterized by light microscopy using Giemsa or hematoxylin-eosin stains, may be birefringent on polarizing microscopy, or can be visualized directly by electron microscopy.\textsuperscript{1,3,9}

Several methods have been suggested for the rapid screening of serum samples for cryoglobulin formation, but these methods have not been well standardized and have not gained general acceptance. Rapid screening methods include detection of light scattering by turbimetry measurements or laser nephelometry, and the induction of an increase in precipitate formation by making a sample hypotonic following mixing with an equal volume of distilled water.\textsuperscript{10-12} These methods may have particular merit for the evaluation of type III cryoglobulins because these cryoproteins are often present at the limits of visual detection (ie, 0.001 to 0.005 mg/mL) and may require up to 7 days of observation before they can be characterized.

**QUANTITATION OF CRYOGLOBULINS**

Following the detection of a precipitate, some effort should be made to show that it is soluble at 37°C. This can be carried out by comparing the sample with a separate aliquot kept at 37°C or by warming the cryoprecipitate. The latter procedure may be inadequate if only small amounts of cryoprecipitate are present, if the sample is hemolyzed, or in conditions (eg, chronic intravascular coagulation) in which increased levels of fibrin occur.

A survey of large clinical reference laboratories reveals considerable variation in methods used for washing cryoglobulins and for quantitation. A minimum of 3 to 6 washes is usually required. In our laboratory, washing is carried out repeatedly with 10 volumes of phosphate-buffered saline until the cryoprecipitate is free of nonspecifically adherent serum proteins, which is determined by the lack of a precipitin line on testing in double-diffusion in agar using an antiseraum to serum albumin. An alternative technique for laboratories utilizing this methodology may be to compare the ratio of γ-globulin to albumin peaks in the solubilized cryoprecipitate to a parallel serum sample analyzed by capillary zone electrophoresis.\textsuperscript{14} If there is a question of residual contamination with red blood cells or fibrin, the cryoprecipitate is resolubilized by warming to 37°C for 1 hour, centrifuged warm at 3000 rpm for 10 minutes, and the supernatant allowed to cryoprecipitate before quantitation.

For the cryocrit, a volume of initially warm serum is observed at 4°C for the time period determined to be optimal for cryoprecipitation, centrifuged cold as described, and the percent total volume occupied by the pellet is determined by visual inspection. Since the cryocrit is not assessed on rigorously washed cryoglobulin, may be affected by the processing of serum, and is not standardized as to volume, tube size, or conditions for centrifugation, it should not be considered an index of disease activity when comparing different patients. However, the procedure is convenient, rapidly available, and inexpensive.

Protein assays (eg, optical density at 280 nm, Folin or Bradford assays) have the advantage of greater sensitivity than the cryocrit and are usually carried out on protein (visible or not) that is adherent to the bottom of the tube following washing and centrifugation. Some laboratories report any value above 0.001 mg/mL as being “positive,” and others will compare the protein determination to a so-called normal range (usually 0 to 60 μg/mL). Other laboratories determine a cutoff by comparison with control sera, and still others use historical controls derived from the literature. The latter values may be based on studies carried out almost 30 years ago, in which cryoglobulin screening was performed on the sera of patients with various types of cutaneous vasculitis and results were compared with cryoprecipitates found in “normals.”\textsuperscript{15} More recent studies have recorded levels in normals below 20 μg/mL.\textsuperscript{12,16} Some of these normal cryo-
globulins were characterized sufficiently to demonstrate that they were type III and immune complexes.15 Two additional factors may confound the interpretation of cryoglobulin quantitation. First, in some cryoglobulins as much as a third of the protein may be of nonimmunoglobulin origin.17 This problem is particularly of concern for the quantitation of type III cryoglobulins and is in part a function of limitations imposed by the low protein concentrations obtained, loss of material with repeated washing, and overlap with normal values; it may also be a function of the presence of proteins, such as C1q, other complement components, fibronectin, and other cryoprecipitagogues that may be present in blood.1 Second, a significant percentage of cryoprotein may not redissolve on repeated rewarming and cryoprecipitation, and may require, for example, 0.1 mol/L acetic acid to aid solubilization. This loss of starting material may vary considerably when different cryoproteins are compared, most likely reflecting a variety of mechanisms responsible for the phenomenon of cryoprecipitability.9

Low-level (ie, <1% to 2% cryocrit or <500 μg/mL) cryoglobulin quantitations reported by a laboratory need careful consideration with regard to the patient under study. For example, although the level of the cryoglobulin reflects the clinical manifestations of disease only to a limited extent, much higher levels would be expected in patients known to have (or strongly suspected to have) myeloma or Waldenström’s disease, or in those who have high levels of serum immunoglobulins, an M-spike, hyperviscosity, or specific symptomatology indicative of occlusive vasculopathy.

FURTHER CHARACTERIZATION OF CRYOGLOBULINS
The next step in the workup of patient sera for cryoglobulinemia is detailed characterization and typing of the cryoprecipitate. This step involves review of relevant serum studies that may have a bearing on classification and immunochemical analyses performed on rigorously washed cryoprecipitate isolated from an adequate volume of serum.

SERUM STUDIES
Relevant serum studies for type I and type II cryoglobulins include the following:

Serum Protein Electrophoresis.—The presence of an M-spike is consistent with a type I or type II cryoglobulin and dictates additional evaluations of gammopathy, including immunofixation analysis of concentrated urine.

Serum Immunofixation.—Immunofixation is needed for the characterization of an established M-spike and is also indicated in the absence of a visible M-spike on routine serum protein electrophoresis.

Quantitative Immunoglobulins.—Quantitative immunoglobulin studies are of value with several regards in the further evaluation of cryoglobulinemia. (1) Both monoclonal and polyclonal increases in IgM may be seen, and can be defined as such by comparison with results obtained by immunofixation. A monoclonal IgM is more precisely measured on serum protein electrophoresis than by direct quantitation (see “Pitfalls in Testing Serum for Cryoglobulinemia”). A monoclonal IgM gammopathy in conjunction with a type I or type II cryoglobulin mandates a clinical evaluation for macroglobulinemia or underlying lymphoproliferative disease. In addition, long-term studies have shown that some patients only subsequently develop clear-cut multiple myeloma or lymphoproliferative disease, sometimes after many years of symptoms.6 Polyclonal or oligoclonal elevations in IgM are common among patients with type II cryoglobulinemia associated with HCV infection. (2) Depressed levels of normal immunoglobulin isotypes may suggest decreased synthesis, as is seen in most cases of multiple myeloma, or may reflect loss of protein, as may result from nephrotic range proteinuria or protein-losing enteropathy. Additional studies are necessary to evaluate these possibilities.

Serum Viscosity.—Evaluation of serum viscosity may be indicated on the basis of markedly elevated levels of monoclonal IgM or clinical symptomatology suggestive of hyperviscosity. Viscosity measurements using the Wells-Brookfield viscometer (Brookfield Engineering Laboratory, Stoughton, Mass) are superior to those obtained with the Ostwald-100 viscometer for the evaluation of cryoglobulinemic sera because they can be performed at variable temperatures (eg, 37°C, room temperature, and 4°C) to demonstrate temperature-dependent changes that may reflect changes in blood rheology in the microcirculation.

OPTIONAL STUDIES
Optional studies for types II and III cryoglobulins include the following:

Complement Levels.—Screening for complement levels is carried out by quantitating antigenic levels of C3 and C4 and determining total hemolytic complement (CH50). Very low or undetectable levels of C4 with normal or relatively normal levels of C3 are often seen in type II cryoglobulinemia and may suggest this diagnosis or a need for further workup for HCV infection. Low complement levels may also be seen in association with type II cryoglobulinemia complicating Sjögren’s syndrome or with type III cryoglobulinemia in patients with rheumatoid vasculitis or systemic lupus erythematosus.

Rheumatoid Factor Activity.—Immunoglobulin M antiglobulin activity can be assessed by agglutination reactions, such as latex fixation, or, more commonly, by nephelometry. Both type II and type III cryoglobulins may be associated with rheumatoid factor activity, either as a consequence of the nature of the mixed cryoglobulin or because of an underlying disease such as rheumatoid arthritis or Sjögren’s syndrome. A very high titer of rheumatoid factor activity, especially in the patient who does not clearly have rheumatoid arthritis, needs to be evaluated for macroglobulinemia.

PITFALLS IN TESTING SERUM FOR CRYOGLOBULINEMIA
Each of the serum studies previously discussed may be influenced by artifacts that arise from ex vivo cryoprecipitation after blood is drawn. Consequently, if a cryoglobulin is suspected, serum should be kept warm, and tests should be carried out at 37°C. Rheumatoid factor activity may be missed if the majority of IgM antiglobulin is cryoprecipitable and is lost during processing19; in HCV infection associated with cryoglobulinemia, the titer of rheumatoid factor activity may be significantly influenced by conditions of cold storage.20 Monoclonal protein may be missed on routine electrophoresis, and quantitation of immunoglobulins may be artifactually low if most of the cryoglobulin has precipitated prior to measurement. Quantitative measurements of immunoglobulin levels by nephelometry may also be inaccurate because they are not
standardized against monoclonal proteins. Cryoprecipitation apparent as a colloid suspension can adversely affect the reliability of light-scattering measurements to quantify complement, immunoglobulin, or rheumatoid factor activity. Cryoglobulins may precipitate at the point of origin when protein electrophoresis is carried out in the cold, creating the illusion of an M-spike.21 Lastly, complement measurements should be carried out whenever possible with serum kept at 37°C to minimize ex vivo complement activation by cryoprecipitable, as well as noncryoprecipitable, immune complexes.

**TYPEING OF ISOLATED CRYOGLOBULINS**

Methods for the typing of isolated cryoglobulins include the following:

**Immunofixation.**—Immunofixation is currently the method of choice for the accurate assessment of clonality in serum and for the typing of cryoglobulins. Greater use of immunofixation in reference laboratories in recent years is probably responsible in part for the higher relative incidences of type II cryoglobulins reported in large series from several referral centers.4,22 Although results correlate well with those obtained by immunoelectrophoresis, immunofixation is faster and easier to interpret. Its sensitivity permits the detection of small amounts of monoclonal proteins in the serum, even in the face of a normal serum protein electrophoresis, and it is particularly useful for the definition of monoclonal immunoglobulins in type I and type II cryoglobulins, the latter of which are almost invariably IgM-k.23

**Immunoblotting.**—Cryoglobulins can be fractionated on composite agarose polyacrylamide gels and probed with antisera to immunoglobulin heavy- and light-chain determinants.24 This method can be performed on as little as 10 μg of material and is useful for the distinction of type II from type III mixed cryoglobulins.25 Immunoblotting combined with immunofixation may be particularly effective for the demonstration of the oligoclonality of each component of the complex.26,27 Reverse immunoblotting using recombinant antigens may also be useful for the definition of specific antibody reactivities in a cryoglobulin, as has been seen in those developing during the course of HCV infection.25

**Two-dimensional Gel Electrophoresis.**—Limited studies have shown that isofocusing may be a sensitive technique for the identification of clonality in mixed cryoglobulins, particularly when gels are run under reducing conditions, the position of κ light chains is defined, and the pattern found for cryoglobulins of interest is compared with a normal pool of heavy and κ light chains.28 Methods such as composite or 2-dimensional gel immunoblotting may prove to be particularly effective in demonstrating transition forms between type II and type III cryoglobulins, and possibly in the evolution of the latter to the former over time.

**Capillary Zone Electrophoresis.**—This method offers the advantage of sensitivity and automation for the characterization of large number of samples. Similar to immunoblotting techniques, it may be particularly suitable for the characterization of cryoproteins at the lower limits of detection using more standard techniques or for serial studies as an index of disease activity. Subtraction analysis of the γ-globulin curve before and after cryoprecipitation can be used as an alternative to protein and immunoglobulin determinations for the quantitation of cryoglobulins.14,29

**HCV-RELATED CRYOGLOBULINEMIA**

An association between HCV infection and the syndrome of purpura, arthralgias, renal disease, and neuropathy that was described initially as “essential” mixed cryoglobulinemia (MC) was first reported in 1990, about a year following the cloning of the virus. This association accounts for approximately 60% to 80% of cases of MC. Conversely, the incidence of cryoglobulinemia in patients with chronic HCV infection (ie, chronic hepatitis without the clinical features listed) has been variously reported as between 13% and 54%.30 Consequently, the evaluation of a patient for HCV infection has become an integral part of the workup of cryoglobulinemia.

Several aspects of HCV infection are relevant for the proper interpretation of laboratory testing for cryoglobulinemia: (1) many chronically infected HCV patients have minimally or intermittently elevated liver function abnormalities, so that normal liver function test results (especially if the patient is on corticosteroids) should not discourage a search for this association if indicated clinically; (2) both type II and type III cryoglobulins may occur in HCV infection, although almost invariably those found in patients with the syndrome of MC are found to be IgM-k/IgG mixed cryoglobulins with selective V-region gene usage31,32; (3) there is little evidence of any association between HCV and gammapathy in the absence of cryoglobulinemia or with type I cryoglobulins; (4) immunologic manifestations that are prevalent in HCV infection include IgM-containing immune complexes, cold-dependent activation of complement, rheumatoid factor activity in serum (~70%), antitissue antibodies (40% to 50%), lymphocytic sialoadenitis (~50%),33 and anticardiolipin antibodies (~20%), the last—similar to the situation in human immunodeficiency virus infection—rarely associated with clinical thrombosis.34

Hepatitis C virus is detected in clinical practice by immunologic testing (most commonly by third-generation enzyme immunoassays with confirmation by recombinant immunoblot analysis) or by detection of viral RNA. Viral RNA may be quantitated by either of 2 commercial methods currently available, namely, the Amplicor competitive polymerase chain reaction assay, which detects viral RNA down to 700 Eq/mL, and the Chiron branched DNA (bDNA) assay, which does not require the reverse transcription step or polymerase chain reaction, but is only sensitive down to 2.0 × 10⁵ Eq/mL.35 Both methods may be applied to isolated cryoglobulins, and some evidence has indicated that both antibody to the virus and viral RNA (more so the latter) may be concentrated in isolated cryoprecipitates.36,37 Immunologic testing for HCV infection is complicated by the fact that there are a number of genotypes of the virus, the relative incidence of which may vary with the geographic area being sampled. The virus circulates in infected patients as quasispecies, which may vary significantly over time and with therapy. This is a function of a high mutation rate (10⁻³ to 10⁻⁴ base substitutions per genome site per year), which leads to changes in amino acid sequence in relevant viral antigens, and immunologic reactivity, which may not be fully apparent on standard assays. In addition, there may be differences between serum and peripheral blood mononuclear-associated or cryoprecipitate-associated quasispecies.38 Finally,
levels of virus may vary by at least 7 logs over time, correlating to some extent with fluctuations in liver function tests. Hepatitis C virus testing in patients suspected or known to have cryoglobulinemia may be quite unreliable, largely due to loss of antibody or HCV RNA during processing; occasional instances have been described in which polymerase chain reaction quantitation was felt to be insignificant until measurements were carried out on the isolated cryoprecipitates.30

A phenomenon that has been called cold-dependent activation of complement (CDAC) is prevalent in HCV-infected persons.40 In CDAC, the level of CH50 is low after cooling to 4°C overnight; the level of C4 protein is normal even though hemolytic C4 is low. Both CDAC and MC may cause complement activation as an ex vivo artifact. Cold-dependent activation of complement was found in 28 (41%) of 69 HCV-infected sera and in no sera infected by hepatitis B virus. More than half of serum samples affected by CDAC show cryoprecipitation, and 35% of samples with cryoprecipitation also showed CDAC. Cold-dependent activation of complement appears to represent a complement abnormality that may correlate somewhat with liver damage and is particularly prevalent in the setting of HCV infection.41 It should be considered in the interpretation of complement protein determinations in patients infected with HCV. The mechanism responsible for this phenomenon is not understood, but may be consequent to HCV antibody–monoclonal rheumatoid factor complexes with differing stoichiometry.

Recent studies have indicated a potentially important relationship between HCV, cryoglobulinemia, and low-grade non-Hodgkin’s lymphoma, which may suggest a broader role for cryoglobulin determinations in lymphoproliferative diseases than has been appreciated previously.6 Of patients with the syndrome of MC, 7% to 39% may develop lymphomas, sometimes after long periods of follow-up. An additional relationship has been suggested by an increased representation of type II MC cross-reactive idiotypes.42 The association of HCV with MC led to surveys of the prevalence of HCV, cryoglobulinemia, and non-Hodgkin’s lymphoma in a number of centers in Italy, where both HCV and MC are endemic. Of 2274 Italian cases of B-cell non-Hodgkin’s lymphoma, 434 (19.1%) were HCV-positive; 41.2% of those analyzed were associated with cryoglobulin production.6 This approximately 20% incidence of HCV infection in non-Hodgkin’s lymphoma has been confirmed in studies from Japan and from 2 centers in the United States.43

**SERIAL STUDIES IN CRYOGLOBULINEMIA**

Until testing for cryoglobulinemia becomes standardized, serial evaluations cannot be recommended as a guide of disease activity or the efficacy of therapy. There is only a poor correlation between laboratory parameters, such as cryoglobulin level, antoglobulin titer, or depressed C4 levels, and clinical symptomatology when different patients are compared. There may be considerable changes in the composition of mixed cryocomplexes over time as IgM levels fluctuate with activity of disease, decrease with treatment, or if patients become hypogammaglobulinemic. The effects of such changes on thermal amplitudes, stoichiometry of complexes with cooling, or the physical properties of cryoglobulins have only been analyzed to a limited extent.6 However, cryoglobulin levels have been found to correlate with response to treatment with modalities such as plasmapheresis, cytotoxic agents, and interferon alfa.44,45 These studies were carried out using a single reference laboratory or highly standardized techniques for collection and processing to ensure reproducibility and prevent loss of cryoprecipitate during handling. The value of prepheresis and postpheresis quantitation of cryoglobulin or immunoglobulin (particularly IgM) levels, or of HCV RNA levels, in the prediction of long-term clinical outcome remains to be determined.

**CONCLUSIONS**

Cryoglobulin testing should be carried out only for specific clinical or laboratory indications and should be interpreted in the context of a careful assessment of monoclonal gammapathy or rheumatologic disorder. The single most important variable confounding standardization is the frequently improper separation of warm serum from other blood elements and casual transport of samples to the laboratory performing quantitation and typing. Most significant cryoglobulins are apparent within a day of refrigeration. Criteria for detection and quantitation include the removal of nonspecifically adherent serum proteins from the precipitate by serial washing and resolubilization with rewarming. A wide variety of methods for quantitation are available, many of which have limited value and are not applicable to low levels of type III cryoglobulins. Characterization of cryoglobulins as type I, II, or III has some utility with regard to specific clinical disorders, notably those recently associated with HCV infection.

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**References**