Analytical Considerations in the Investigation of Mixed Cryoglobulinemia

To the Editor:

Schnabl et al. (1) recently presented a clinical case study of a patient with mixed cryoglobulinemia. It is important that such cases receive attention because cryoglobulinemia is often overlooked in diagnostic workups; however, several technical points regarding the analytical aspects of investigation require clarification.

In their protocol for the investigation of cryoglobulins, the authors advocate the use of the “cryocrit” as a means of quantifying and typing the cryoglobulin in question. Their comments about “cryocrit” are, in fact, confusing. In their Fig. 1, they indicate that the cryocrit should be performed on “washed precipitate,” but in the Discussion they state that the cryocrit is the “(precipitate volume)/ (total volume of serum sample).” It is not clear whether they are recommending centrifugation of the washed precipitate or the original chilled serum sample. Cryocrit is at best a crude semiquantitative method to estimate the amount of cryoprotein present. Standardization and QC procedures are lacking.

Native cryoprecipitate contains both coprecipitants and contaminants, including other serum proteins, viral particles, and bacteria (2). Washing in a cold buffered saline solution may reduce the contamination, but it will also cause the loss of a variable amount of cryoglobulin. Given that most mixed cryoglobulins are present only in milligram concentrations, quantification by this method is unlikely to yield accurate results.

From a theoretical standpoint, the use of the cryocrit is rather dubious because it assumes that different cryoproteins pack and sediment with equal volumes. In particular, experience at this institution suggests that cryogels have very unpredictable sedimentation characteristics. The cryocrit is highly dependent on the speed and duration of centrifugation. Packing of the gel after centrifugation is often irregular, with a variable volume of serum being retained around its surface, giving rise to difficulties in washing and necessitating careful manipulation.

Brouet et al. classified cryoglobulins into 3 main types, depending on their immunologic composition (3). Type I cryoglobulins are single monoclonal immunoglobulins (usually IgM or IgG), whereas type II and type III (mixed) cryoglobulins are immune complexes consisting of either monoclonal or polyclonal IgM, respectively, directed against polyclonal IgG.

The authors state that a “diffuse γ region visible after protein electrophoresis... suggested the presence of polyclonal γ-globulins.” The clonality of cryoglobulins can only be determined immunochemically. It is unclear, therefore, whether the patient’s cryoglobulin was definitively “typed.” The absence of an “M band” upon protein electrophoresis does not exclude the possibility of a monoclonal component being present. Furthermore, the inclusion of the cryocrit in Fig. 1 as a determination of the “type” is also misleading.

It is important to emphasize that the aim of typing is not solely to distinguish purely monoclonal from mixed cryoglobulinemia (i.e., type I from type II/III). Differentiating type II from type III has important prognostic implications, because a considerable number of patients with type II cryoglobulinemia may eventually develop non-Hodgkin lymphoma or other lymphatic/hepatic malignancies (4).

Also to be emphasized is that the cryoglobulin concentration is not an index of disease severity; low concentrations of mixed cryoglobulin may be associated with life-threatening presentations, whereas patients with relatively high concentrations can be asymptomatic. Other investigators have reported an apparent inverse relationship between the cryocrit and vasculitis (2). This situation is further complicated by the fact that many healthy individuals have detectable amounts of cryoglobulins; therefore, detection is not necessarily indicative of disease and needs to be interpreted in the specific clinical context of each case.

Overall, it is questionable whether quantification by such crude methods adds value to the diagnostic investigation, because there is no evidence that it informs clinical decision-making or patient management. Despite this fact, these crude quantitative measurements remain firmly entrenched in current practice (5).

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References


Letters to the Editor
to a difference in opinion on the usefulness of estimating cryoglobulin quantities. Although some investigators reported no relationship between cryoglobulin concentration and the severity of symptoms and disease activity (2), cryoglobulin concentrations have, nevertheless, been found to correlate with response to treatment with plasmapheresis, cytotoxic agents, and/or interferon α (3). Moreover, we did indicate in our report that cryocrit does not differentiate type 1 and type 2 cryoglobulinemia, and we recommended that serum protein electrophoresis and immunofixation should be conducted on resublubilized cryoprecipitate (4). Certainly, laboratories that do not have specialized equipment or a test in place to screen for cryoglobulins should consider at minimum estimating the cryocrit.

In Reply

E. Smith has pointed out that the use of cryocrit in the investigation of cryoglobulinemia has analytical and clinical limitations, an observation with which we concur in general. The emphasis of our case study, however, was not to make a judgment on which technique should be used, but rather to focus on the importance of including cryoglobulin analysis in the differential and to address the need for standardization between laboratories. We reported a schema of sample collection and analysis that merely reflects a practice that many laboratories still conduct today. Vermeersch et al. reported that up to 37% of the 140 surveyed laboratories included cryocrit or other estimates (e.g., total protein) in their report. The lack of standardization of practice appears, at least in part, to be related to a difference in opinion on the usefulness of estimating cryoglobulin quantities. Although some investigators reported no relationship between cryoglobulin concentration and the severity of symptoms and disease activity (2), cryoglobulin concentrations have, nevertheless, been found to correlate with response to treatment with plasmapheresis, cytotoxic agents, and/or interferon α (3). Moreover, we did indicate in our report that cryocrit does not differentiate type 1 and type 2 cryoglobulinemia, and we recommended that serum protein electrophoresis and immunofixation should be conducted on resublubilized cryoprecipitate (4). Certainly, laboratories that do not have specialized equipment or a test in place to screen for cryoglobulins should consider at minimum estimating the cryocrit.

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To allow better evaluation for the clinical and laboratory community, Apple suggested a scorecard for cardiac troponin assays (1), with the value of the troponin assay being based on 2 criteria. The main criterion is the imprecision of the assay at the 99th percentile of a reference population. A good assay was defined as one with a 10% CV at the population’s 99th percentile, a clinically usable assay was defined as one with a CV between 10% and 20% at that percentile, and a CV > 20% at this cutpoint was deemed unacceptable. Apple further differentiated assays on their ability to detect troponin in healthy control individuals. We have concerns with some aspects of his scorecard.

Our major point of disagreement with Apple is that he uses the 99th percentile of a reference population and the imprecision of the assay for assessing assay quality but gives no consideration to the clinical value of that assay. This latter point is of particular importance. For example, Venge et al. used samples from the FRISC (Fast Revascularisation during Instability in Coronary Artery Disease) study to compare the performance of the Liaison cardiac troponin I assay (DiaSorin) in unstable coronary artery disease (2). The Liaison assay showed good imprecision, with a 10% CV at 0.027 μg/L and a 99th percentile reference population value of 0.041 μg/L. This assay would have been qualified as acceptable; however, comparison with another troponin assay, the Access AccuTnI from Beckman Coulter, showed that the Liaison assay missed about 10% of patients with unstable angina and an increased risk of death or acute myocardial infarction within 6 months. More recently, a large prospective cohort study of patients presenting with chest pain to the emergency department showed similar results (3). In this study, assays that were more sensitive for troponin I showed an improved diagnostic performance for acute myocardial infarction compared with a so-called standard assay (Roche fourth-generation troponin T assay). Assays with superior diagnostic performance in the recent cohort study were the Abbot Architect, the Siemens Centaur Ultra, and the Roche Elecsys high-sensitivity troponin T assays. Assays that were less sensitive included the fourth-generation troponin T assay; however, according to Apple’s scorecard, the Abbot Architect assay is ranked as “clinically usable” at his level I designation, similar to the rank of the fourth-generation Roche Elecsys troponin T assay. The use of assay imprecision defined as a 10% CV and the 99th percentile of healthy individuals falls short in judging assay performance.

A better way forward to compare assay performance might be to use patient serum pools with different cardiac troponin concentrations, as was done in a 2004 study (4). In this study, the Abbott AxSYM assay was unable to measure troponin in the pool with the lowest concentration, as were other analyzers at the time: the Immuno 1 analyzer (Bayer Diagnostics), the Vidas analyzer (bioMerieux), the Dimension RXL analyzer (Siemens), the Opus analyzer (Dade Behring), and the Vitros ECi analyzer (Ortho Clinical Diagnostics). In contrast, other analyzers measured detectable troponin down to the pool with the lowest concentration. Clearly, these findings illustrate the different analytical sensitivities of the different assays. This consideration translates into the recognition of additional patients at risk.

In his scorecard, Apple uses the 10% CV as an important point of assay differentiation. However, there is no evidence to support the use of the 10% CV; in fact, the opposite is true. There currently is a wealth of evidence showing that in the setting of acute coronary syndrome, troponin concentrations corresponding to assay CVs well above the 10% CV are informative for a worse prognosis (5).

What is the way forward? Although we agree that assays can and should be scored objectively on their technical characteristics, we also believe that assays can and should be compared for their clinical performance. Useful information for comparing troponin assays includes clinical cohort studies that use chest pain patients in the emergency department, patients with a high pretest probability for unstable angina, and patient populations from the glycoprotein IIb/IIIa inhibitor trials. Additional clinically relevant information can be obtained from the exchange of patient pools and measuring troponin in these pools with different analyzers and assays. This matter needs further debate.
References


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