### The Interfering Component in Cardiac Troponin I Immunoassays: Need for Further Experimental Evidence

*To the Editor:*

I read with great interest the report by Eriksson et al. *(1)*, discussing the existence of a frequently occurring blood component that interferes with immunoassays measuring cardiac troponin I (cTnI) by use of antibodies against epitopes in the central part of the molecule. As a main indicator of the assay accuracy, the authors studied the analytical recovery of a purified preparation of human cardiac ternary TnI-TnT-TnC complex (from HyTest Ltd., Turku, Finland) added to different samples obtained from healthy individuals and from patients with acute coronary syndrome, using assays that recognize different cTnI epitopes.

By definition, recovery refers to the ability of an analytical method to measure an analyte correctly when a known amount of it is added to authentic samples *(2)*. Estimation of recovery can therefore be an effective means of obtaining accuracy information because it tests whether the method can measure the analyte in the presence of all other compounds contained in the matrices of authentic samples. Unfortunately, there were some pitfalls in the recovery experiments performed by Eriksson et al. *(1)* as the criterion for demonstrating the presence of a circulating inhibitory component. They did not consider the characteristics of the material they add to the biological fluids. Addition of pure analyte, such as the native, tissue-derived troponin complex, in a complex matrix in vitro is obviously an artificial approach. One cannot be sure that the physical or chemical state of the cTnI in the HyTest preparation, e.g., its solubility, is the same as that for cTnI in vivo. The purification procedure can lead to partial alteration of cTnI structure with the consequence that the probability of significant effects by the complex matrix becomes high, which might affect the immunoassay reaction *(3, 4)*.

Recently we performed recovery experiments using a similar material to test the reactivity to various cTnI forms in a newly introduced cTnI immunoassay *(5)*. Although the cTnI recovery was good [mean (SE), 92.2 (10.1)%; four dilutions] when the stock solution of the material was serially diluted with appropriate buffer, the recovery decreased [73.7 (7.2)%; four dilutions] when the material was diluted with a commercially available cTnI-free diluent (cat. no. 8TFS; HyTest). In this case, we simply considered that the physicochemical properties of the material might differ significantly according to the different diluents used.

As in other studies *(6)*, we have also seen problems in the stability of the material. After thawing, we noticed a significant loss in the specific activity within a few hours. This could be an additional confounding factor.

In their discussion, Eriksson et al. *(1)* recognized that the "inhibitory" effect was "somewhat more prominent with tissue-derived ternary troponin complex (calibration material) than with endogenous cTnI forms and more evident in EDTA plasma than in serum"; thus, they indirectly considered the possible influence of the type of added cTnI and of the sample matrix on the obtained results *(1)*.

Another limitation of their study was that the authors support their conclusions by claiming higher sensitivity in the early phase of infarction by use of a cTnI immunoassay supplementing a monoclonal antibody with an epitope in the N-terminal region of cTnI and one with an epitope in the C-terminal region to the midfragment cTnI antibodies. Apart from the anecdotal report of only two cases showing a different behavior, the use for comparison of three cTnI assays that previously have shown only moderate performance for precision and sensitivity at low-range cTnI concentrations should be considered inadequate *(7, 8)*.

I agree that a comparison study may be the only practical way to definitively assess the accuracy of the different analytical approaches evaluated for cTnI measurement, but method-comparison studies should compare results obtained with the new proposed analytical approach with those obtained with a reference-quality immunoassay for which, in addition to the use of antibodies against the central part of cTnI, excellent performance for analytical sensitivity has been documented *(9, 10)*. As also reported in the Information for Authors of this journal *(11)*, it is desirable to test 100–200 different samples from patients with acute coronary syndrome who have been selected to present a range of cTnI values that includes those likely to be encountered in routine application, i.e., samples collected during development of minor and major myocardial injuries, comprising very early and late phases after symptom onset. Difference plots are recommended to show dependence of the differences on cTnI concentrations.

In conclusion, as one of the coauthors of the IFCC recommendations on the use of antibodies that "preferably recognize epitopes that are located in the stable part of the cTnI molecule and are not affected by complex formation and other in vivo modifications" *(12)*, I concur with Eriksson et al. *(1)* on the importance of obtaining detailed and incontrovertible information on the possible occurrence in the blood of an interfering factor that may negatively affect cTnI measurements obtained with many commercial assays, whose manufacturers have endorsed the above-mentioned recommendation *(13)*. However, more and possibly definitive experimental evidence should be collected that permit identification and isolation of this component, which should then be used to directly test the interference in cTnI immunoassays.

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## Factor V Leiden in Blood Donors in Baghdad (Iraq)

*To the Editor:*

Human coagulation factor V is a 330-kDa single-chain glycoprotein that plays an important role in the coagulation pathway. After its activation by thrombin and factor Xa, activated factor V (Va) forms an essential part of the prothrombinase complex, which catalyzes the conversion of prothrombin to thrombin in the presence of calcium and a phospholipid membrane *(1)*. Activated protein C (APC) inactivates factor Va and requires factor V as a cofactor in the APC-mediated inactivation of factor VIII *(1, 2)*. Resistance to APC action leads to increased risk of thrombosis and is mostly attributable to a single point mutation in the factor V gene (factor V Leiden) with a G-to-A substitution at nucleotide 1691 *(3)*.

Several studies have demonstrated the high prevalence of factor V Leiden in some Middle Eastern countries, with suggestions that the eastern Mediterranean basin maybe the site where this mutation arose ~21 000–34 000 years ago *(4, 5)*. Despite the fact that Iraq lies within this region, no such prevalence studies have been reported on the incidence of factor V Leiden in that country. We report here the results of the first such study.

Between September 21, 2002, and February 22, 2003, we evaluated a total of 100 blood donors attending the national blood bank, the only blood-banking facility serving the population of Baghdad City and the surrounding regions in central Iraq. Samples were collected, through venipunctures separate from those used for donation, from 10 donors randomly selected by use of random numbers (between 1 and 40, generated for each day) from among the first 40 attending the bank on 10 alternate Saturdays throughout the study. During the first nine collection sessions, one randomly selected donor declined participation, and one sample was hemolyzed; therefore, on the last session, 12 random samples were taken. The study was approved by the Council of the College of Medicine-University of Baghdad, and informed consent was obtained from all donors evaluated.

All included patients were males, consistent with the donation pattern in Iraq, with ages ranging from 18 to 52 years (median, 30.5 years). A second-generation APC resistance test was performed on all included patients with a STACLOT® APC-R reagent set (Diagnostica Stago) with modifications; 100 μL of prediluted test plasma (1:10 in factor V-deficient plasma) was incubated for exactly 3 min at 37 °C with 100 μL of *Crotalus viridius helleri* venom, after which 100 μL of APC/calcium chloride was added, and the clotting time was recorded. The clotting times ranged from 39 to 111 s, with a mean (SD) of 70.4 (13.5) s. The cutoff point for "resistant" cases was 48 s as determined by logarithmic transformation of clotting times, after exclusion of outliers (>3 SD above or below the mean) and calculation of the mean − 1.96 SD. All included donors had their DNA extracted, amplified, hybridized to wild- and mutant-type DNA probes, and detected by enzyme immunoassay according to the instructions of the manufacturer (ViennaLab).

Four cases (4%) were below the calculated cutoff point of the second-generation APC test and were thus considered resistant. Three cases (3%) were found to be heterozygous for factor V Leiden by DNA studies (all of whom were APC resistant with clotting times of 43, 45, and 48 s). The remaining case, resistant by second-generation APC resistance tests (clotting time, 39 s) was a noncarrier of factor V Leiden, This may indicate that an alternative mutation involving the factor V gene maybe responsible for APC resistance in this particular case *(6)*.

The prevalence rate of 3% in Iraqi blood donors, although it is much lower than the prevalence rates of 14.2%, 13.6%, and 12.25% reported in Lebanon, Syria, and Jordan, respectively *(4, 7)*, and to a lesser extent than the rates in Turkey (7.4%) *(8)* and Iran (5.5%) *(9)*, is comparable to the prevalence of 2.5% reported in